Antibacterial activity of clove *Eugenia caryophllata* extracts on bacteria isolated from animal and human

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DEDICATION

To the late Prof. Abdel Dawi..

To my Parents … brothers and sisters

With love..

To my Colleagues and friends

With best respect.

Acknowledgment

First of all my thanks and praise to almighty Allah, the Beneficent, the Merciful, for giving me health and strength to accomplish this work. Then I wish to express my indebtedness and sincere thankfulness to my supervisor Dr. Suliman Mohammed El Hassan for his keen guidance, valuable assistance, advice and encouragement. Indeed, his generous help and support is greatly appreciated.

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Abstract

Eugenia caryophllata is a plant which is believed by Sudanese herbalists to have antimicrobial effect. This plant has been tested in the present study to investigate their *in vitro* potential effect against nine Gram positive and Gram negative bacterial species.

The tested organisms were *Staphylococcus aureus, Streptococcus pyogense, Streptococcus pneumoniae, Bacillus cereus, Corynebacterium renale, Pseudomonas spp, Klebsiella spp, E. coli* and *Salmonella spp*.

The plant was extracted with three solvents, water, methanol and petroleum ether. Methanol was used to extract the polar compounds and the petroleum ether was used to extract the fatty compounds.

The extracts were tested by three methods, agar dilution method, impregnated filter paper disc method and cup plate method. Water, methanol and petroleum ether extracts of the clove were examined at three concentrations, 10%, 20% and 30%.

In the disc method and the cup plate method the extract was prepared and examined in three concentrations of 10%, 20% and 30% and the test organisms were cultured on DST medium.

Water extract of *Eugenia caryophllata* inhibited the growth of *Staphylococcus aureus, Bacillus cereus, Corynebacterium renale, Klebsiella spp, E.coli, Pseudomonas spp* and *Salmonella spp* at all concentrations.

Methanolic extract of *Eugenia caryophllata* inhibited the growth of *Staphylococcus aureus, Bacillus cereus, Corynebacterium renale, Klebsiella spp, E.coli, Pseudomonas spp* and *Salmonella spp* at all concentrations. But the petroleum inhibited the growth of *Bacillus cereus, E.coli* and *Klebsiella spp* at high concentration (30%).

The three extracts of *Eugenia caryophllata* examined in this study at different concentrations were not effective against *Streptococcus pyogense* and *Streptococcus pneumoniae*.

Water extract of *Eugenia caryophllata* was found to be more effective as antimicrobial agent than the methanolic extract and petroleum ether extract.

Methanolic extract of *Eugenia caryophllata* was found to be more effective as antimicrobial agent than the petroleum ether extract.

In the present study *Eugenia caryophllata* (Clove) was found to be a good antimicrobial agents. More work on *Eugenia caryophllata* (Clove) may prove it is a powerful antimicrobial agent.

الخلاصة

يعتقد العشابون السودانيون أن لنبات القرنفل تأثير مضاد للبكتريا. اختبرت الدراسة الحالية هذا النبات معمليا لمعرفة تأثيره على 9 أنواع من البكتريا الموجبة لصبغة الجرام و السالبة لصبغة الجرام. أستخدم الماء و الميثانول و الايثر البترولي كمذيبات. أستخدم الميثانول كمذيب لاستخلاص المواد القطبية من النبات كما أستخدم الأيثر البترولي كمذيب لاستخلاص المواد الدهنيه.

في هذه الدراسة اختبرت المستخلصات النباتية بثلاث طرق ، طريقه تخفيف الاجار ، طريقه أقراص ورق الترشيح المشبعة وطريقه تقطيع آبار الاجار . وأختبر مستخلص الماء ومستخلص الميثانول ومستخلص الأيثر البترولي لنبات القرنفل على ثلاثة تراكيز 10%، 20%، 30%.

في طريقة الاقراص وفي طريقة تقطيع الاجار تم تجهيز المستخلص وأختبر بالتراكيز الأتيه 10%،20%،30% وحضنت البكتيريا المراد اختبارها علي أجار DST .

في هذه الدراسة وجد ان مستخلص القرنفل عن طريق الماء ثبط نمو غالبية البكتيريا بالتراكيز الثلاثة و هذه البكتيريات هي *Staphylococcus aureus, Bacillus cereus, Corynebacterium renale, Klebsiella spp, E.coli, Pseudomonas spp* and *Salmonella spp.*

مستخلص المواد القطبيه للقرنفل ثبط نمو غالبيه البكتريا بالتراكيز الثلاثة هذه البكتريات هي: *Staphylococcus aureus, Bacillus cereus, Corynebacterium renale, Klebsiella spp, E.coli, Pseudomonas spp* and *Salmonella spp.*

و لكن مستخلص المواد الدهنيه ثبط نمو البكتيريا عند التراكيز العالية فقط والبكتيريات هي

Bacillus cereus, E.coli and *Klebsiella spp*.

وجد أن كل المستخلصات لنبات القرنفل لم يكن لها تأثير مضاد علي

Streptococcus pyogense, Streptococcus pneumoniae.

في هذه الدراسة وجد أن الماء كمستخلص لنبات القرنفل أكثر فعالية كمضاد للبكتيريا مقارنه بمستخلص الميثانول و الايثر البترولى.

المواد القطبية للقرنفل أكثر فعالية كمضاد للبكتريا مقارنه بمستخلص المواد الدهنيه لنفس النبات .

في هذه الدراسة وجد أن نبات القرنفل فعال كمضاد للبكتريا وبمزيد من الدراسة قد يصبح من المضادات البكتيرية المستخدمة في علاج الأمراض البكترية .

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Introduction

Many cultures around the world have strong believes on the use of many different plants as medicine which has rendered some culture, up to date, almost completely dependant on the use of plants in medicine rather than common commercial medicinal products (Stockwell, 1988).

Historically, plants have played an important role in medicine. Medicinal plant, in general, have been an issue of great controversy through the history of mankind. For early peoples, they came easily to hand, and were intricately connected to diet and healing. Through observation and experimentation, they learned which plants promoted health and well-being. Actually, the use of plants and herbs as medicine has almost become a differentiating aspect between first world and third world countries and cultures. This seems ironic as many of the modern medicinal products are actually derived or extracted from plants (Cowan, 1999).

The Clove (*Eugenia caryophllata*) is an aromatic plant, which grows in the tropical regions of Africa, Asia, and South America. There are reports that clove oil may relieve gum and tooth pain and may be useful as a topical antiseptic in mouth wash.

Generally it is believed that clove is antiseptic and has anesthetic properties. Clove and clove oil combat some microbial infection, relieve nausea, vomiting, improve digestion, ease arthritis inflammation and hiccups.

Commission E(German regulatory agency for herbs) approved clove oil for use as an antiseptic.

It has long been believed in the Sudanese herbal, and medicinal plants culture that have antimicrobial effects, This plant is actually used by the Sudanese herbal for treatment of many bacterial infection. There are local use of plants in the Sudan to treat abdominal pain, diarrhoea, wounds and mouth pain.

This lead us to study the effects of plants, against representative Gram positive and Gram negative bacteria. These types of pathogenic organisms and disease they cause have been among the difficult to handle due to their resistance to many of the common antibiotics.

In this study, water, methanol and petroleum ether extracts of clove will be examined for antimicrobial activity against various bacterial species. Also the bacterial growth inhibitory effects of these preparation will be compared with antibiotics commonly used for treatment of bacteria.

CHAPTER ONE
LITERATURE REVIEW

1.1 Bacteria and Disease they caused:

1.1.1 The genus Staphylococcus:

Staphylococci are Gram-positive, spherical cells occuring singly in pairs or clusters. They are natural habitat from birth to death. Man lives in an environment that is rarely free of *S.aureus*. The human nose is the natural reservoir of the organism (Briody, 1974). The skin and large intestine represent additional source for the contamination of the atmosphere with *S.aureus* (Buchanan and Gibbons,1974; Melville and Russel, 1975 ; Talaro and Talaro, 1993).

Traditionally, staphylococci have been divided into two groups according to their ability to clot blood plasma (coagulase reaction). Coagulase-positive staphylococci strains have been subdivided according to the animal host, serotype and phagotype, in general, names for the new subspecies of coagulase-positive staphylococci have not been proposed (Cohen, 1982; Cohen, 1986; Thomas, 1988). In contrast, Coagulase-negative staphylococci have been further differentiated according to their biochemical characteristic and cell wall chemistry.

1.1.1.1 Staphylococcus infection:

Staphylococci are perhaps the best example parasites that have great pathogenic potential, yet are able to live in symbiotic balance with their hosts. In spite of their ability to produce serious, life threatening disease, pathogenic staphylococci are present on skin or mucous membrane of all human (Jensen and Waright, 1998). Staphylococci are part of the normal microflora of the

upper respiratory tract (Kumar and Clark, 1996). Staphylococci cause a wide range of infection both in man and animals, which are summarized in three groups (Davis, Mecarty and Wood, 1973 ; Melville and Russell, 1975 ; Jawets, Melnick and Adel berg, 1980 ; Cohen, 1982 ; Talaro and Talaro, 1993).

Group A : Suppurative (Skin) :

- Impetigo, contagious
- Epidermal necrolysis
- Abscess (deep tissue)

Group B : Systemic :

-Bacteraemia (from abscess, wounds, burn)

-Osteomyelitis

-Pneumonia

1.1.1.2 Pathogenicity of *Staphylococcus aureus*:

Staphylococcus aureus is usually a secondary infection in patient with chronic lung disease (Macsween and Whaly, 1992). *S.aureus* causes boils, pustules, impetigo, infection of wounds, ulcers and burns, osteomyelitis, mastitis, septicemia, meningitis, pneumonia and pleural empyema (Cheesburgh, 2000).

1.1.2 The genus Streptococcus :

Streptococci are Gram-positive cocci, non-motile. aerobic, catalase and oxidase negative (Barrow and Feltham, 1993).

1.1.2.1 Pathogenicity of *Streptococcus pneumoniae* :

Streptococcus pneumoniae causes lobar pneumonia, bronchitis, meningitis, bacteraemia, otitis, sinusitis and conjunctivitis. Severe infections can occur in the elderly and those already in poor health or immune suppressed. In tropical and developing countries, *S.pneumoniae* is a major pathogen it is also a common cause of childhood pneumonia (Cheesburgh, 2000).

1.1.2.2 *Streptococcus pyogense* :

The most important of the hemolytic streptococci as cause of human and animal infection. On blood agar, *Streptococcus pyogense* produce small colonies about 1 mm in diameter surrounded by an area of clear haemolysis.

1.1.2.3 Pathogenicity of *Streptococcus pyogense* :

Streptococcus pyogense causes tonsillitis, pharyngitis, peritonsillar abscess, scarlet fever, otitis media, cellulites, impetigo , necrotizing fascitis, septicemia and glomerulonephritis (after skin or throat infections). The most common and most typical infection caused by *S.pyogense* is an acute sore throat called tonsillitis (Colle *et al*., 1989) .

1.1.2.4 Streptococcal throat infection:

Streptococci appear to have special predication for the lymphatic system, and in the upper respiratory tract the initial location of the infection is in the lymphoid tissue of the pharynx (Dubos and Hunch ,1965). Streptococcus sore throat infection is an infection caused by a particular strain of streptococcus bacteria (Peter, 2002). The action of the streptococcus bacteria in the throat or on the tonsils stimulates an inflammatory response

and the lyses of leucocytes and erythrocytes (Lansing *et al*.,1999). From the throat streptococci may spread to the surrounding tissue causing otitis media, mastoditis and suppurative adenitis. It may also cause meningitis (Geo *et al*., 1995). Streptococcal pneumonia is rare and usually secondary to influenza or measles (Macsween and Whaly, 1992).

Streptococcus pneumoniae remain a major cause of morbidity and mortality in undeveloped parts of the world , and resistance to common antibiotics is wide spread (Tomaz ,1997 ; Crook and Spant ,1998 ; Charpentoer and Tuomanen , 2000 ; David *et al*., 2003). The most common route of entry of *Streptococcus pyogense* is by the upper respiratory tract where the primary infection is established , usually in the throat , but only a proportion of infected individuals develop tonsillitis in pharyngitis (Colle *et al*., 1989).

1.1.3 The *Corynebacterium renale*:

This bacterium is diphtheroid, Gram positive rods. Glucose is slowly acidified and all strains are catalase positive. Members of the *C. renale* group inhabit the lower genital tract of cattle and sometimes other ruminant. Occasionally they are implicated in urinary tract disease of sheep, horse, dogs and non human primates. Organisms pass between animals by direct and indirect contact.

In cattle, the process is an ascending urinary tract infection, beginning with cystitis, which proceeds to ureteritis and pylonephritis. Chronic infections progress to debilitation and death due to uremia (Coyle and Lipsky, 1990).

1.1.4 The genus Escherichia:

Escherichia is a genus of the family enterobacteriaceae and types Eschericheae. Like many other enterobacteria contains numerous serotypes some of which are associated with certain infections in man and animals, some are particularly associated with diarrhoeal disease while other causes a variety of extra intestinal infection (Orskov and Orskov, 1976).

1.1.4.1 General characteristic of *Escherichia coli* :

E.coli is Gram negative rod-shaped, oxidase negative, catalase positive. *E.coli* has a world wide in distribution. Many *E.coli* are part of normal flora of the intestinal tract of human and animal. Some species are free living occuring in soil , water and vegetation (Carter, 1985).

1.1.4.2 *E.coli* infections :

Escherichia coli causes various disease in human and animals including several types of diarrhoea (watery diarrhoea, cholera like diarrhoea, watery to dysentery like diarrhoea and mucoid diarrhoea), urinary tract infections sepsis, hemorrhagic uraemic syndrome and meningitis in man (Nataro and Kaper, 1998). In animal *E.coli* causes profused watery diarrhoea in most animal species, odema, hemorrhagic colitis, septicemia and mastitis (Basamat, 2003).

1.1.5 The genus *Pseudomonas*:

It is Gram-negative rods, motile, catalase positive, oxidase positive. Most members of the genus pseudomonas live in soil and water (Quinn *et al*., 2000).

1.1.5.1 Pseudomonas infections:

Characteristics feature of *Pseudomonas pneumoniae* infection is bacterial invasion of the subsequent hemorrhage or thrombosis and then pulmonary infection (MacSween and Whaly, 1992). The lungs of children with cystics fibrosis are very susceptible to infection with *Pseudomonas aeruginosa* (Colle *et al*., 1989).

1.1.6 The genus *Klebsiella*:

Klebsiellae are Gram-negative rods, non motile, usually capsulated, catalase positive, oxidase negative (Barrow and Feltham, 1993).

1.1.6.1 Klebsiella infection :

Although members of this group are lacking pathogenicity, some of the types are associated with mastitis, respiratory infections and urogenital infections of animals. *Klebsiella spp* display an opportunistic pathogenic ability in the respiratory tract, the urinary system or as the cause of surface infections and are associated with both endemic and epidemic infections in hospital (Colle *et al*., 1989). *Klebsiella pneumoniae* is present in the respiratory tract; cause a small proportion of bacterial pneumonias (Geo *et al*., 1995). It is opportunist pathogen, it may produce pyogenic lesions like abscess, infection of wound, or respiratory tract (Satish, 1995).

1.1.7 The genus *Salmonella*:

The salmonellae are short Gram-negative rods, motile except *S.pullorum* and S.gallinarum, and non capsulated (Bergey's 1974). The reservoir for Salmonella is the intestinal tract (Quinn *et al*., 2000).

1.1.7.1 Disease caused by *Salmonella species*:

Salmonellosis (paratyphoid) is a disease of all animals caused by a number of different species of salmonellae and it manifested clinically by one of three major syndrome : a peracute septicemia and acute enteritis. In salmonellosis there are often dysentery with whole blood being passed in large clots, and abdominal pain, in pregnant cows there are abortion. In caprine the form of disease is acute enteritis.

Salmonella is also a bacterium that is wide spread in the intestines of birds, reptiles and mammals. It can spread to human via a variety of different foods of animal origin. The illness it causes salmonellosis which is typically includes fever, diarrhoea and abdominal cramps. In persons with poor underlying health or weakened immune systems, it can invade the blood stream and cause life-threating infections (CDC, 2003). Salmonella also causes typhoid fever which is caused by *S.typhi* (Hassan and Gumaa, 1985).

1.1.8 *Bacillus*:

Bacillus species is a large Gram positive, endospore-forming rod. Members of the genus bacillus are catalase positive, aerobic or facultatively anaerobic and motile with the exception of *B.mycoides* and *B. anthracis* (Quinn *et al*., 2000).

1.1.8.1 Disease caused by *Bacillus species*:

Anthrax is one of the most important disease caused by bacillus. This disease is caused by *Bacillus anthracis,* it is a zoonotic disease, worldwide in distribution but it is common in Africa, Asia, South America and Eastern Europe (Blood *et al*., 1985).

Bacillus cereus has been incriminated as a cause of gangrenous bovine mastitis and abortion in cows. In human it has been implicated in food poisoning (Carter, 1986).

1.2 Chemotherapy of bacterial infection:

1.2.1 Anti microbial agents:

Antimicrobial drugs are divided in two classes, based upon their general effect on bacterial population. These are bacteriocidal and bacteriostatic. Ehrlich found that the a arsenical compound, arsphenamine, was selectively toxic for *Terponema pallidum*, this was the first a long series of drugs to be synthesized in the laboratory. A number of years later (Domagk,1935) showed that the red dye prontosil was effective in the treatment of streptococcal infections. Later it has been discovered that it was due to sulfonamide derived from prontosil. The success of this drug stimulated a search for related compounds and resulted in the synthesis of effective compound which is the sulfonamide group (Carter *et al*., 1986).

1.2.2 Antibiotics :

The antibiotics are a group of complex organic chemicals which are produced initially by microorganisms during their growth and which in minute quantities have detrimental effects on other organisms (Brander *et al*., 1977). Pasteur and Joubert (1877) first reported that air borne contaminants had lethal effect on culture of *B.anthracis*. In 1929, Fleming observed that a fungus *Penicillium notatum*, was strong inhibitor to the growth of Staphylococci, when present on culture plate. In 1940, Chain, Florey, and associated succeeded in obtaining a preparation from penicillium. After the discovery of penicillin an extensive search of antibiotics was began (Carter *et*

al., 1977). In 1949, Waksman and Lechevalier isolated a soil organism, *Streptomyces fradiae* which produced an antibiotic that in crude contained an antifungal compound (Fradine) and a groups of antibacterial substances that were labeled neomycin (Rolinson and Steven, 1961).

1.2.3 Problems of antibiotics:

Firstly broad spectrum antibiotics cause disturbance in digestion when used orally due to their effect on microflora. Secondly the development of resistance among bacterial population exposed to antibiotics received great deal of attention both in human and veterinary medicine (Brander *et al*., 1977). Resistance can be caused by a variety mechanisms such as the presence of enzymes that inactive the antimicrobial agent; a mutation in the antimicrobial agents target, which reduces the binding of the antimicrobial agent; the presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent; reduce uptake of the antimicrobial agent; active efflux of the antimicrobial agent; and overproduction of the target of the antimicrobial agent. In addition, resistant may be caused by unrecognized mechanisms (Tenover and Unger, 1993). Thirdly antibiotics have been used in the therapy of animal and it may be harmful to human, and it may cause problems in the control of human diseases (Brander *et al*., 1977). Finally some antibiotic are very expensive so in recent years, medicinal plant represented a primary health source for the pharmaceutical industry (Brander *et al*., 1977).

1.3 Medicinal plants :

1.3.1 Uses of plants in medicine:

The use of plants for the treatment of various diseases is universal and has been practiced by many people since ancient times. Hippocrates in the late fifth century B.C. mentioned 300 to 400 medicinal plants (Schultes,1678).In the first century A.D. Discorides wrote *De Materia Medica*, a medicinal plant catalog which became the prototype for modern pharmacology.

The use of medicinal plants varies from disease to another. The Neem tree (*Azadirachta indica*), for example, has been known in Asia for along time. The sun-dried seeds of the plant are used by Indians to control pests in house as well as stored cereal grains, and as a detergent resembling shampoo, for the removal of lice from the head (Jotwani and Srivastova,1984; Ibrahim, 1990). The Neem tree has also been found to have an antimicrobial effect (Khalid *et al*., 1989). In African countries, the ripe fruit of *Balanites aegyptiaca* , Laloub is a popular medicinal plant and is used as a purgative and anthelmintic (Oliver, 1986 ; Nakhla , 1990). *Cassia senna* and *C.italica*, both of the family *Leguminasceae* were the first cassia species reported in literature for their therapeutic value as purgatives. The chemical constituents in the ripe pods of these plants are emodin, aloe, and chrysophanol (Friedrich and Steftem, 1973).

Cucurbita maxima and *C.peppo* seeds kernel (Kousa) are used in many African countries including the Sudan as well as Europe and Asia as anthelmintic for tape worms and as diuretic and the scraped fruit pulp is applied as a poultice to burns, boils and swelling or as cooling application for headache (Oliver, 1986; Mohamed, 1992). Plant constituents possessing antibacterial activity include flavonoids in *Polygonum senegalense*, phenol chloropherin (Lewis and Elvin Lewis, 1977), in *Clorophora excelsa* thymol

and euganol in (Jain and Jain , 1972). The volatile oils of black peeper, clove, geranium, nut meg oregano and thyme were assessed for antibacterial activity against 25 different genera of bacteria. These include animal and plant pathogens, food poisoning and spoilage bacteria. The volatile oils exhibited considerable inhibitory effects against all the organisms under test while their major components demonstrated various degree of growth inhibition (Dorman and Deans, 2000).

1.4 The plant: *Eugenia caryophllata*, Clove (Fig.1)

The clove is an aromatic spice that grows as evergreen tree in the tropical regions of Asia, Africa and South America. It belongs to:

Family: Myrtaceae

Genus: *Eugenia*

Species*: Eugenia caryophllata*

Scientific/ medical names*: Syzgyium aromaticum, Caryophyllum, Eugenia aromatica*

Common name: Clove

Arabic name : قرنفل

1.4.1 Historical background:

During the Handy nasty (207 B.C. to 220 A.D.) those who addressed the Chinese emperor were required to hold cloves in their mouth to mask bad breath. Traditional Chinese physicians have long used the herb to treat indigestion, diarrhoea, hernia and ring worm, as well as athlete's foot and other fungal infection. India's traditional Ayurvedic healers have used clove since ancient time to treat respiratory and digestive infection.

Clove first arrived in Europe around the 4th century A.D. as a highly coveted luxury. The medical German herbalist used clove as part of anti-gout mixture. Once clove became easily available in Europe, it was prized as a

treatment for indigestion, flatulence, nausea , vomiting and diarrhoea. It was also used to treat cough, infertility, warts, worms, wounds and toothache. Early American Eclectic Physicians used clove to treat digestive complaints and added it to bitter herb-medicine preparation to make them more palatable. They were also the first to extract clove oil from the herbal buds. They used it on the gums to relieve toothache. Contemporary herbalist recommends clove for digestive complaints and its oil for toothache.

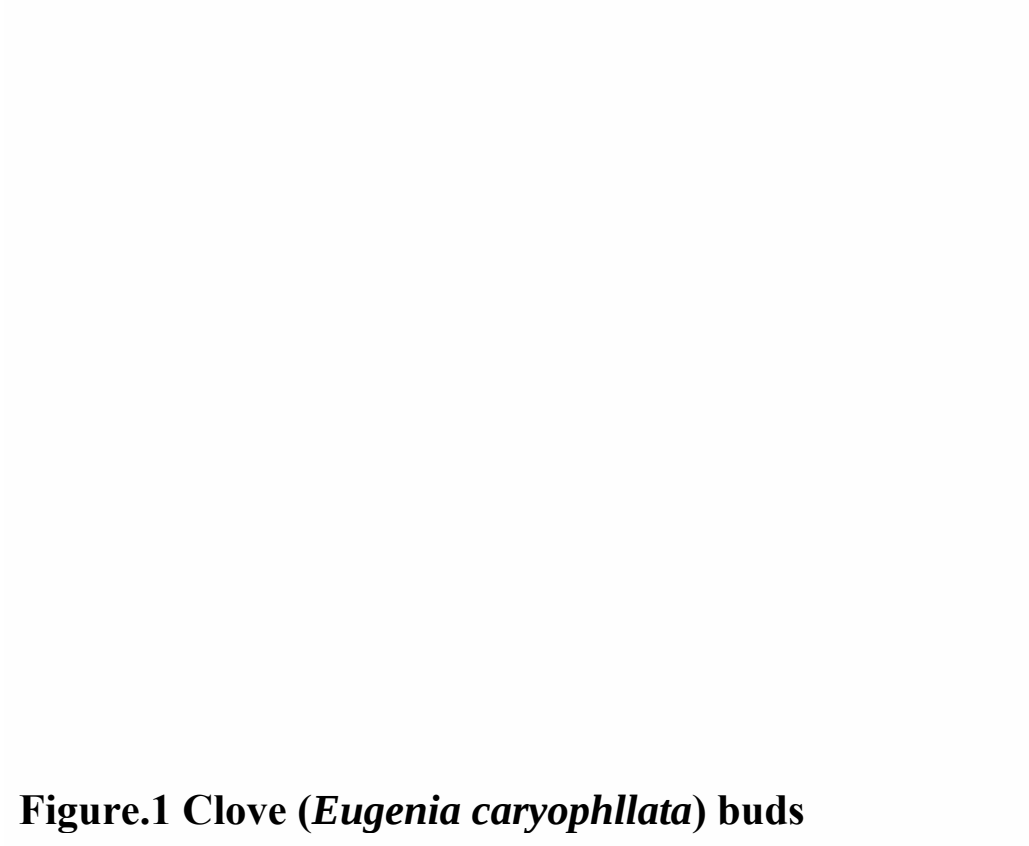


Figure.1 Clove (*Eugenia caryophllata*) buds

1.4.2 Uses of clove in medicine:

Clove kills intestinal parasites and exhibits broad antimicrobial properties against fungi and bacteria supporting its traditional use as a treatment for diarrhoea, intestinal worms and other digestive ailments. Clove are said to have antiseptic and anesthetic properties. Clove and clove oil combat fungal infection, relieve nausea and vomiting, improve digestion, fight intestinal parasites, stimulate uterine contraction, ease arthritis inflammation, stop migraine headache, and ease symptoms of colds and allergies. Some dentists and patients reported that clove oil may relieve gum and tooth pain and may be useful as a topical antiseptic in mouth wash (Fetrow and Avila, 1991). The microbiological quality of drinking water is a major public health priority in development countries. Various parts of the plants, i.e. cloves, seeds and fruits, they are used against *E.coli* in drinking water (Blech, Guillemin , Baure and Hartemann, 1991). Clove are generally considered safe, although a relatively small number of people may be allergic to eugenol (Zheng and Kenny, 1992).

1.4.3 Uses of essential oils in medicine:

The antimicrobial properties of essential oils have been known for many centuries. In recent years (1987-2001), a long number of essential oils and their constituents have been investigated for their antimicrobial properties against some bacteria and fungi. The classical methods commonly were used for the evaluation of essential oils antibacterial and antifungal activities. The agar diffusion methods and the dilution methods as well as turbidimetric and impedimetric monitoring of microorganisms growth in the presence of tested essential oils are described, essential oils of used against microorganisms in

the research are thyme, oregano, mint, cinnamon, salvia and clove (Kalemba and Kunicka, 2003).

Some essential oils such as clove, eucalyptus, lavender, mint, myrrh and mille folia are used for treatment against inflammatory disease including arthritis, rheumatism, acne skin allergy and ulcers (Darshan and Doreswamy, 2004). The antibacterial activity of 11 essential oils from aromatic plants against the food borne pathogen *Bacillus cereus*, the essential oils of clove, nutmeg, mint, oregano, cinnamon, sage and theme used against *Bacillus cereus* (Valero and Salmeron, 2003).

1.4.4 Other uses of clove and essential oils:

Essential oils, odorous and volatile products of plant secondary metabolism, have a wide application in folk medicine, food flavoring and preservation as well as fragrance industries (Kalimba and Kunicka, 2003). The essential oils of clove, cinnamon, pimento and rosemary were found to be most active in inhibition of some bacteria involved in meat spoilage.

A relationship was found between the inhibitory effect of essential oils and the presence of euganol and cinamaldehyde (Quatora *et al.*, 1997).

Essential oils of spices and herbs (thyme, origanum, mint, cinnamon, salvia and clove) were found posses the strongest antimicrobial properties on some bacteria and fungi of human and food-borne bacteria and fungi (Kalimba and Kunicka, 2003).

Today, cloves are used in baking and cooking and as ingredient in perfumes, cigarettes and toothpaste (Fetrow and Avila, 1991).

Table 1: Antimicrobial effect of some essential oils :

Organism	Condition	Essential oil
Candida albicans	Yeast infection affecting mouth, vagina skin folds, bowel.	Petit grain, Sandalwood, Thyme, Cinnamon bark.
Clostridium perfringens	Affects intestinal tract causing blood poisoning, food poisoning, gas gangrene	Clove bud
Corynebacterium diphtheriae	Airborne-causes diphtheria toxins harmful to cardiac and nerve tissue	Tea tree
Diplococcus pneumoniae	Associated with pneumonia.	Clove bud, Eucalyptus, Thyme, Cinnamon bark.
Enterobacter aerogenes	Intestinal infection producing gas.	Thyme
Enterococci spp.	Colon infection	Clove bud, Thyme, Cinnamon bark
Beta hemolytic streptococci	Inhibits, destroys red blood cells	Thyme, Lavender
Escherichia coli	Gastro-intestinal infection	Thyme, Tea tree, Cinnamon bark
Klebsiella spp.	Respiratory, intestinal, Genito-urinary tract infections	Thyme, Cinnamon bark

Mycobacterium tuberculosis	Airborne-tuberole infection of lungs, Lymph nodes and meninges	Hyssop
Proteus spp.	Urinary infection	Thyme
Pseudomonas aeruginosa	In soil-pathogenic-in pus from wounds, Urinary tract infections	Clove bud, Thyme, Tea tree
Salmonella pullorum	Food poisoning septicemia	Cinnamon bark, Thyme
Salmonella typhi	Typhoid fever	Tea tree
Sarcina spp.	Infection of the skin	Thyme
Staphylococcus albus	Skin infection	Clove bud, Thyme, Cinnamon bark
Staphylococcus aureus	Boils, abscesses, impetigo (bulbous) and non bulbous	Tea tree, Sandal wood, Cinnamon bark
Yersinia enterocolitica	Intestinal infection	Thyme (Thymol)

***Adapted from:**

Carson, Cookson, Riley (1995), Deans and Svoboda (1989), Juven, Kanner, Schaved and Weisslowcz (1993) and Valnet (1982).

CHAPTER TWO

2. MATERIALS AND METHODS

2.1. Sterilization:

a. Flaming:

It was used to sterilize glass slides, cover slips, needles and scalpes.

b. Red heat:

It was used to sterile loop wires, points and searing spatulas by holding them over Bunsen burner flame until became red-hot.

c. Hot air oven:

It was used to sterilize glasswares such as test tubes, graduated pipettes, flasks, forceps and cotton swabs. The holding period was one hour and oven temperature was 180C°.

d. Steaming at 100C°:

Repeated steaming (Tyndallization) was used for sterilization of sugars and media that could not be autoclaved without deteriment effect to their constituents. It was carried out as described by Cruckshank *et al*. (1975).

e. Moist heat (autoclave):

Autoclaving at 121°C (151b/inch^2) for 15 minutes was used for sterilization of media and plastic wares.

Autoclaving at 115°C (10Ib/inch) for 10 minutes was used for sterilization of some media.

2.2 Reagents and indicators:

2.2.1 Reagents:

2.2.1.1 Alpha-naphthol solution:

Alpha-naphthol is a product of British Drug House, London (BDH) .This reagent was prepared as 5% aqueous solution for Voges Proskauer (VP) test.

2.2.1.2 Potassium hydroxide:

It was used for Voges Proskauer test and was prepared according to Barrow and Feltham (1993) as 4% aqueous solution.

2.2.1.3 Hydrogen peroxide:

This reagent was obtained from Agropharm limited , Buckingham .It was prepared as 3% aqueous solution and it was used for catalase test.

2.2.1.4 Methyl red:

It was prepared by dissolving methyl red (0.04 g) in ethanol (40ml). The volume was made to 100 ml with distilled water. It was used for methyl red test (MR).

2.2.1.5 Tetra methyl-p-phenylene diamine dihydrochloride:

This was prepared in a concentration of 3% aqueous solution and was used for oxidase test.

2.2.1.6 Nitrate test reagent:

Nitrate test reagent consist of two solutions and they were prepared according to Barrow and Feltham (1993) .Solution A was composed of 0.33% sulphanilic acid dissolved by gentle heating in 5N-acetic acid. Solution B

was composed of 0.6% dimethyelamine-alfa -naphthylamine dissolved by gentle heating in 5 N-acetic acid.

2.2.1.7 Kovac's reagent:

This reagent composed of 5g para-dimethylaminobenzaldehyde, 75ml amyl alcohol and 25ml concentrated hydrochloridic acid. It was prepared as described by Barrow and Feltham (1993) by dissolving the aldehyde in the alcohol by heating in water bath, it was then cooled and the acid was added. The reagent was stored at 4°C for later use in indole test.

2.2.2 Indicators:

2.2.2.1 Andrade's indicator:

It composed of acidic fuchsin 5g, distilled water 1L and N-NaOH 150ml .The acidic fuchsin was dissolved in distilled water, then the alkali solution was added, mixed and was allowed to stand at room temperature for 24h with frequent shaking until the color changed from red to brown.

2.2.2.2 Bromothymol blue:

It was obtained from BDH. The solution was prepared by dissolving 0.2g of the bromothymol blue powder in 100 ml distilled water.

2.2.2.3 Phenol red:

It was obtained from Hopkins and William 1td ,London. It was prepared as 0.2% aqueous solution.

2.2.3 Collection of blood for enriched media:

Blood for enriched media was collected aseptically into sterile flask containing glass beads by veino puncture of the jugular vein of healthy sheep kept for this purpose. The blood was defibrinated by shaking the sterile flask containing glass bead during and after collection.

2.4 preparation of media:

2.4.1 Nutrient broth:

Thirteen grams of nutrient broth powder (Oxoid) were added to one liter of distilled water, mixed well and distributed in 3 ml amount into clean test tubes, then sterilized by autoclaving at 121°C for15 minutes.

2.4.2 Peptone water:

Fifty grams of peptone water powder (Oxoid) were added to one liter of distilled water, distributed in 3 ml amount into clean test tubes and sterilized by autoclaving at 121°C for 15 minutes.

2.4.3 Peptone water sugars:

Peptone water sugar medium was prepared as described by Barrow and Feltham (1993). It contained peptone water 900ml, Andrade's indicator 10 ml, sugar solution 10 ml and distilled water 90 ml. The pH of peptone water was adjusted to 7.1 – 7.3 before the addition of Andrade's indicator. The complete medium was mixed well, then distributed into portion of 2 ml into clean test tubes containing inverted Durham's tube and sterilized by autoclaving at115°C for 10 minutes.

2.4.4 Nitrate broth:

The medium used was prepared as described Barrow and Feltham (1993). Potassium nitrate 1g was dissolved in 1 liter of nutrient broth, distributed in 5 ml amount into test tubes and sterilized by autoclaving at 115°C for 20 minutes.

2.4.5 Glucose- Phosphate Medium (MR-VP Test medium):

This medium was prepared according to Barrow and Feltham (1993). Peptone 5g and 5g powder buffer solution were added to 1 liter of distilled water, dissolved by steaming till dissolved completely, filtered and pH was adjusted to 7.5. Then 5g of glucose were added , mixed well, distributed into clean test tubes and sterilized by autoclaving at 115°C for 15 minutes.

2.4.6 Nutrient agar:

To one liter of nutrient broth (Oxoid) 15g of agar were added , dissolved by boiling , sterilized by autoclaving at121°C for 15 minutes. Then cooled to about 50°C and distributed in 15 ml amount per sterile petri dish plate. The poured plates were left to solidify at room temperature on leveled surface.

2.4.7 Blood Agar:

Forty grams of blood agar base № 2 (Oxoid) were suspended in 900 ml of distilled water, dissolved by boiling, mixed and sterilized by autoclaving at 121°C for 15 minutes. Then cooled to about 50°C, defibrinated sheep blood was added aseptically to give final concentration 10%, mixed gently and 15 ml of complete medium was poured into each sterile petri-dish. The poured plates were allowed to solidify at room temperature on flat surface.

2.4.8 Diagnostic sensitivity test Agar:

This medium was supplied by (Oxoid). It consist of protease peptone, veal infusion solids, dextrose, sodium chloride, di-sodium phosphate, sodium acetate, adenine sulphate, guanine hydrochloride, uracil, xanthine and ion agar № 2. Forty grams of the medium were suspended in one liter of distilled water, then brought to boil to dissolve completely, sterilized by autoclaving at 121°C for 15 minutes, then dispended into sterile petri dishes in portions of 15 ml each. The poured plates were left to solidify at room temperature on leveled surface.

2.4.9 MacConkey agar :

Fifty two grams of MacConkey agar (Oxoid) were suspended in one liter of distilled water, brought to boil to dissolve the ingredients completely, then sterilized by autoclaving at 121°C for 15 minutes and poured into sterile petri dishes in 15 ml amount. The poured plates were left to solidify at room temperature on flat surface.

2.4.10 Motility medium- Gragie tube medium:

Thirteen grams of dehydrated nutrient broth (Oxoid) were added to 5g of Oxoid agar No.1 and dissolved in one liter of distilled water. The pH was adjusted to 7.4. This medium was dispended in volume of 5ml into 20 ml test tubes containing the appropriate Gragie tubes, then the medium in the test tubes were sterilized by autoclaving at 121°C for 15 minutes.

2.4.11 Hugh and Leifson,s (O/F) medium :

This medium was prepared as described by Barrow and Feltham (1993).Two grams of peptone powder, five grams of sodium chloride, 0.3g of potassium hypophosphate and three grams of agar were added to one liter of distilled water . Then heated in water bath at 55°C to dissolve the solids . The pH was adjusted to 7.1 and filtered . Then the indicator bromothymol blue (0.2% aqueous solution) was added and the mixture was sterilized at 115°C for 15 minutes . Filtered sterile glucose solution was added aseptically to give final concentration of 1%. Then the medium was mixed and distribute aseptically in 10ml amount into sterile test tubes of no more than 16 mm diameter.

2.4.12 Mannitol salt agar:

One hundred and eleven grams of Oxoid CM85 dehydrated medium were suspended in a liter of distilled water, mixed, steamed to dissolve and then the pH was adjusted to 7.5. It was then autoclaved at 121°C for 15 minutes, cooled and poured into petri dishes. The poured plates were allowed to solidify at room temperature on flat surface.

2.4.13 Glucose phosphate medium:

Peptone 10 grams and K_2HPO_4 5 grams were added to distilled water 1000 ml. Then steamed to dissolve and filtered through a filter pulp and the pH was adjusted to 7.5, after that 5 grams of glucose were added mixed and distributed into test tubes and sterilized at 110°C for 10 minutes .

2.4.14 Nutrient gelatin:

One hundred and twenty eight grams of nutrient gelatin (Oxoid CM 132 a) were hydrated in a liter of distilled water, steamed to dissolve, pH was adjusted to 6.8, distributed in screw-capped bottles and autoclaved at 121°C for 15 minutes.

2.4.15 Starch agar:

Potato starch 10 grams, distilled water 50 ml were mixed with nutrient agar 1000 ml. The starch was triturated with water to smooth scream and added to the molten nutrient agar, mixed and sterilized by autoclaving at 115°C for 10 minutes cooled and poured in petri dishes. The poured plates were allowed to solidify at room temperature on flat surface.

2.5 Collection of samples :

Total number of 145 samples were collected from human, sheep, goat, calves, cattle and poultry. The source and number of samples collected are shown in table 2.

The human throat samples were collected from Khartoum North hospital. While the eye and nose samples were collected from healthy people by qualified nurse. The cattle mastatic milk, eye and abscess swab samples were collected from Khartoum North farms and Faculty of Veterinary Medicine clinic.

Tables 2: **Source and types of samples collected from human, sheep, goat, calves, cattle and poultry.**

Animal Species	Source of sample	Samples collected	№. Of samples collected
Sheep	Skin abscess	Swab	10
	Eye	Swab	3
Goat	Mastatic milk	Milk	13
	Mouse	Swab	3
	Diarrhoetic faeces	Faeces	4
	Urine	Urine	6
Calves	Diarrhoetic faeces	Faeces	10
Cattle	Mastatic milk	Milk	20
	Skin abscess	Swab	7
	Eye	Swab	5
Poultry	Diarrhoetic faeces	Faeces	12
Human	Throat	Swab	40
	Eye	Swab	3
	Nose	Swab	6

2.5.1 Sample Transport:

All samples collected were transported on ice in thermos flask to the laboratory for immediate processing and culturing.

2.6 Culture of specimens:

The collected samples were inoculated onto blood agar media. The inoculated plates were then incubated for 24-48 hours at 37°C. After the incubation period the colonies characteristic were observed and smears were made from each type of colony , dried in the air , then fixed by heating , stained by Grams methods and examined under light microscope for cell morphology and staining reaction .

2.7 purification:

All bacteria isolated were purified by several subculturing from single well-separated colony. The purity of the culture was checked by examining Gram stained smear . The pure culture was then used for studying cultural and biochemical characteristics and sensitivity of the isolates.

2.8 Microscopic Examination:

Smears were made from each type of colony on primary culture and from purified colonies , fixed by heating and stained by Gram method Barrow and Feltham (1993). Then examined microscopically under high power . The smear was examined for cell morphology and staining reaction.

2.9 Identification of bacteria:

The purified isolates were identified according to the criteria described by Barrow and Feltham (1993). This included staining reaction, organism

morphology, growth condition, the colony characteristics on different media, haemolysis on blood agar, motility and biochemical characteristics.

2.10 Biochemical methods:

2.10.1 Catalase test:

The test was carried out as described by Barrow and Feltham (1993) . A drop of 3% H_2O_2 was placed on clean slide and a colony of tested culture on nutrient agar was picked by glass rod and added to the drop of 3% H_2O_2. A positive reaction was indicated by production of air bubbles.

2.10.2 Coagulase test:

The test was performed as described by Barrow and Feltham (1993) . To 0.5 ml of 1:10 dilution of human plasma and saline, 0.1 ml of 18-24 hour old culture of the tested organism was added, then incubated at 37°C and examined after 6-24 hour for coagulation. Definite clot formation indicated positive result.

The test was also performed on slide. Two colonies of tested culture were placed on a clean glass slide, emulsified in drop of normal saline and then a loopfull of human plasma was added to the drop of bacteria suspension. Appearance of coarse visible clump was recorded as positive result.

2.10.3 Oxidase test:

The method of Barrow and Feltham (1993) was used. Strip of filter paper was soaked in 1% solution of tetra methyl-p-phenylene diamine dihydrochloride and dried in hotair oven and then placed on clean glass slide by sterile forceps. A fresh young tested culture on nutrient agar was picked

off with sterile glass rod and rubbed on the filter paper strip. If a purple color developed within 5-10 seconds, the reaction was considered positive.

2.10.4 Oxidation-fermentation (O/F) test:

The test was carried out as described by Barrow and Feltham (1993). The tested organism was inoculated by stabbing with straight wire into duplication of test tubes of Hugh and Leifson,s medium. To one of the test tubes a layer of melted soft paraffin oil was added to the medium to seal it from air. Both inoculated tubes were incubated at 37°C and examined daily for fourteen days. Yellow color in open tube only indicated oxidation of glucose; Yellow color in both tubes showed fermentation reaction and blue or green color in open tube and yellow color in the sealed tube indicated production of alkali.

2.10.5 Sugar fermentation test:

The test was carried out as described by Barrow and Feltham (1993). The peptone water sugar was inoculated with organism under the test, incubated at 37°C and then examined daily for several days. Acid production was indicated by appearance of reddish color, while gas production was indicated by presence of empty space in the inverted Durham's tubes.

2.10.6 Indole production test:

Indole production test was carried out as described by Barrow and Feltham (1993). The tested organism was inoculated into peptone water and incubated at 37°C for 48 hour. One milliliter of the Kovacs reagent was run down along side of the test tube. Appearance of pink color in the reagent layer within a minute indicated positive reaction.

2.10.7 Methyl red (MR) test:

Methyl red test was carried out as described by Barrow and Feltham (1993). The tested organism was inoculated into glucose phosphate medium (MR-VP medium) then incubated at 37°C for 48 hour . Two drops of methyl red reagent were added, shaken well and examined. Appearance of red color indicated positive reaction, whereas orange or yellow color indicated negative reaction.

2 .10.8 Voges-Proskauer (VP) test:

The test was performed as described by Barrow and Feltham (1993). The test culture was inoculated into glucose phosphate medium (MR-VP medium) and incubated at 37°C for 48 hour. Three milliliter of 5% alpha-naphthol solution and one milliliter of 40 % potassium hydroxide were added. When bright pink color developed within 30 minutes, the reaction was regarded as positive.

2.10.9 Nitrate reduction test:

The nitrite reduction test was carried out as described by Barrow and Feltham (1993).The test culture was lightly inoculated into nitrate broth and incubated at 37°C for two days. Then 1ml of solution A followed 1 ml of solution B of nitrate test reagent were added. Red color indicated positive reaction that showed nitrate had been reduced. If red color did not develop , powdered zinc was added to see whether there was residual nitrate or not. Red color development indicated that nitrate in medium had been reduced to nitrite by zinc but not by organism, whereas unchanged color indicated nitrate in original medium had been reduced completely and nitrite was further broken down by the organism .

2.10.10 Motility test:

The Craigi tube in semi-solid nutrient agar prepared as described by Cruckshank *et al*, (1975) was inoculated by straight wire. A small piece of the colony of the bacterium under test was picked by the end of the straight wire and stabbed in the center of semi solid agar in the Craigi tube and then incubated at 37°C overnight. The organism was considered motile if there was turbidity in the medium in/ outside the Craigi tube.

2.10.11 Urease test:

The slant surface of urea agar medium was streaked with the test cultures and incubated at 37°C for 24-48 hours. The development of a pink color was indicative of production of NH_3. Negative and weak tests were left for a weak before taking results.

2.10.12 Hydrolysis of gelatin:

Cultures were inoculated by stabbing in nutrient gelatin medium in screw-capped bottle with straight wire loop and incubated at 37°C for 14 days and examined every 2-3 days for liquefaction after cooling at 4°C for half and an hour. Positive and negative controls were set up.

2.10.13 Hydrolysis of starch:

Plates of starch agar were inoculated by streaking with test cultures and incubated at 37°C for 5 days . They were then flooded with iodine, hydrolysis was indicated by clear colorless zones around colonies where there was no hydrolysis the medium turned blue.

2.10.14 Hydrogen sulphate production:

Peptone water was inoculated with test organisms and a lead acetate paper was placed between the cotton plug and tube, incubated at 37°C and examined daily for a week. Blackening of the paper was considered positive.

2.11 *Eugenia caryophllata* extraction methods:

2.11. 1 Water extraction:

Water is almost universal solvent used for extraction of plant ingredient. An amount of 10 grams of *Eugenia caryophllata*, were soaked in 100 ml of distilled water in a sterile flask for 24 hour. The contents of the flask were then filtered. The filtrate was kept at 4°C for later use.

2.11.2 Methanolic extraction:

An empty oven dried, clean, soxhlet flask was accurately weighted . Two grams of ground, oven dried, sample of *Eugenia caryophllata* were weighted out and placed into a soxhlet thimble. The thimble was plugged shut using a piece of cotton wool. The thimble containing the dried sample was then placed in the soxhlet apparatus column which was then assembled and placed on the electric heater. The methanol was added until siphoning started, i.e. distillation. Whenever the siphoning stopped, more methanol was added in order to refill the column. This distillation process was continued for about 6 hours until the empty column was almost full. Then, the column was detached from the apparatus, its contents emptied until about 2-3ml of methanol remained in the flask, and reassembled. The flask was then detached and dried at room temperature for about 1-2 hours then oven dried at 85-100°C until methanol traces remained which took about 2-3 hours. The flasks were then finally placed in open air to cool.

2.11.3 Petroleum ether extraction:

An empty oven dried, clean, soxhlet flask was accurately weighted. Two grams of ground, oven dried, *Eugenia caryophllata* sample was weighted out and placed into a soxhlet thimble. The thimble was plugged shut using a piece of cotton wool. The thimble containing the dried sample was then placed in the soxhlet apparatus column which was then assembled and placed on the electric heater. The petroleum ether (b.p60°-80°C), was added until siphoning started, i.e. distillation. Whenever the siphoning stopped, more petroleum ether was added in order to refill the column. This distillation process was continued for about 6 hours until the empty column was almost full. After this, the column was detached from the apparatus, its contents emptied until about 2-3 ml of ether remained in the flask, and then reassembled. The flask was then detached and dried at 85°-100°C until ether traces remained. This took about 2-3 hours. The flasks were then finally placed in open air to cool.

2.12 *In-vitro* antimicrobial testing:

2.12.1 Agar dilution method:

Water, methanol and petroleum ether extracts of *Eugenia caryophllata* were prepared as described above. The effects of these extracts on growth of Gram positive and negative bacteria were examined. DST medium was sterilized by autoclave at 121°C for 15 minute and allowed to cool to about 50°C. The extract of *Eugenia caryophllata* was then added directly to the DST medium by dissolving in order to establish different concentration of 10%, 20%, 30% and then mixed gently. The complete medium was then poured into petri dishes. The test organisms was then inoculated onto the three different concentrations and incubated at 37°C for 24 hours. The

organism was considered resistant when it grew on the medium containing the extract. The organism was considered sensitive when no growth was noticed on the plate containing the extract.

2.12.2 Disc method:

Filter paper discs of 5mm diameter were used. The discs were impregnated with 10%, 20%, and 30% concentration of each extract. The tested organisms were then cultured onto DST medium free of extract and the impregnated discs were then placed on the agar surface after drying of inoculated plate at room temperature for ½ hour. Inhibition zones around discs was measured in centimeter and then scored as (+) when inhibition zone was 0.5cm ; (++), 1cm ; (+++), 1.5cm; (++++), 2cm; and (-), when no inhibition was noticed.

2.12.3 Cup plate method:

DST agar plate was inoculated with tested organism, then allowed to dry at room temperature for ½ hour. Cups were made in inoculated plate by culturing inoculated DST agar with a sterile cork borer and then the cut agar was removed carefully so that the edges of the circle cup are not lifted. These cups filled with extracts, as described by (Brander and Pugh1977). The concentrations of extracts used were 10%, 20%, 30%. Inhibition zones around the cups was measured in centimeter and then scored as (+) when inhibition zone was 0.5cm; (++), 1cm; (+++), 1.5cm; (++++), 2cm and (-), when no inhibition was noticed.

CHAPTER THREE

3. RESULTS

3.1 Bacterial isolation and identification :

Total number of 145 samples were collected from human, sheep, goat, calves, cattle and poultry. The human throat samples were collected from Khartoum north hospital. While the eye and nose samples were collected from healthy people by qualified nurse. The Mastatic milk, eye, nose swabs, faeces and urine samples were collected from farms and Faculty of Veterinary Medicine clinic.

The bacterial isolates obtained in this investigation were classified on the basis of their cultural characteristics, cell morphology, Gram stain reaction and their biochemical properties as described by Cowan and Steel (1993).

The total number of bacteria isolated was 112, 62 isolates were Gram positive and 50 were Gram negative. The isolated Gram positive bacteria were 31 *Staphylococcus aureus* (28.2%), 15 *Streptococcus pyogense* (13.6%), 11 *Streptococcus pneumoniae* (10.1%), 3 *Bacillus cereus* (2.7%) and 2 *Corynebacterium renale* (1.8%).The isolated Gram negative bacteria were 17 *Pseudomonas spp* (15.4%), 11 *Klebsiella spp* (10.1%), 13 *E.coli* (11.8%) and 9 *Salmonella spp* (8.1%) as shown in Table 3.

Table 3 :Bacteria species isolated from human, sheep, goat, calves, cattle and poultry

Bacteria species	Number of samples examined	Number of isolates	Isolation percentage
S.aureus	110	31	28.2%
St.pyogense	110	15	13.6%
St.pneumoniae	110	11	10.1%
B.cereus	110	3	2.7%
C.renale	110	2	1.8%
Pseudomonas spp	110	17	15.4%
Klebsiella spp	110	11	10.1%
E.coli	110	13	11.8%
Salmonella spp	110	9	8.1%

3.2 Water extraction :

3.2.1 Agar dilution method :

The effect of water extract of *Eugenia caryophllata* on tested bacteria using agar dilution method are shown in Table 4. The three different concentrations of water extract of *Eugenia caryophllata* (10%, 20% and 30%) were examined by agar dilution method.

The three concentrations inhibited all the tested organisms except *Klebsiella spp* which showed a mild growth.

3.2.2 Disc method :

The effect of water extract of *Eugenia caryophllata* on tested organisms using disc method are shown in Table 5, Figure 2.

Bacillus cereus, Pseudomonas spp and *Staphylococcus aureus* were sensitive to 10% concentration of the water extract. The inhibitory zone appeared around the disc measured 1cm in diameter for *Bacillus cereus* and *Pseudomonas spp* and 0.5cm for *Staphylococcus aureus*.

While *Corynebacterium renale*, *Streptococcus pyogense*, *Streptococcus pneumoniae*, *Klebsiella spp*, *E.coli* and *Salmonella spp* were resistant and showed no zone of growth inhibition.

At concentration 20%, the inhibition zone measured 1.5cm for *Pseudomonas* and *Bacillus cereus*, 1cm for *Staphylococcus aureus* and *Corynebacterium renale* and 0.5cm for *Salmonella spp* and *Klebsiella spp* while *E.coli*, *Streptococcus pyogense* and *Streptococcus pneumoniae* showed no inhibition of growth around the disc.

At concentration 30%, the inhibition zone measured 1.5cm for *Pseudomonas spp* and *Bacillus cereus*, 1cm for *Staphylococcus aureus*, *Corynebacterium renale, Salmonella* spp and *Klebsiella spp* while *E.coli*,

Streptococcus pyogense and *Streptococcus pneumoniae* appeared resistant and showed no zone of growth inhibition.

3.2.3 Cup plate method :

The effect of water extract of *Eugenia caryophllata* against tested organisms using cup plate method are shown in Table 6, Figure 3 and 4. At concentration of 10%, the zone of growth inhibition measured 1.5cm for *Pseudomonas spp*, *Bacillus cereus* and *Staphylococcus aureus* and 1cm for *Corynebacterium renale* while *Klebsiella spp, E.coli*, *Salmonella spp*, *Streptococcus pyogense* and *Streptococcus pneumoniae* were resistant and showed no zone of growth inhibition.

At concentration of 20%, the zone of growth inhibition measured 2cm for *Pseudomonas spp*, *Bacillus cereus*, *Corynebacterium renale* and *Staphylococcus aureus*, 1cm for *Klebsiella spp* and *E.coli* and 0.5cm for *Salmonella spp* while *Streptococcus pyogense* and *Streptococcus pneumoniae* were resistant and showed no zone of growth inhibition.

At concentration of 30%, the zone of growth inhibition measured 2cm for *Pseudomonas spp*, *Bacillus cereus*, *Corynebacterium renale* and *Staphylococcus aureus* and 1.5cm for *Klebsiella spp*, *E.coli* and *Salmonella spp* while *Streptococcus pyogense* and *Streptococcus pneumoniae* were resistant and showed no zone of growth inhibition.

Table 4: Sensitivity of Gram positive and Gram negative isolated bacteria to water extract of *Eugenia caryophllata* determined by agar dilution method.

Bacteria species	Sensitivity to water extract		
	At concentration 10%	At concentration 20%	At concentration 30%
Staphylococcus aureus	S	S	S
Streptococcus pyogense	S	S	S
Streptococcus pneumoniae	S	S	S
Bacillus cereus	S	S	S
Corynebacterium renale	S	S	S
Pseudomonas spp	S	S	S
Klebsiella spp	R	R	R
E.coli	S	S	S
Salmonella spp	S	S	S

S= Sensitive (no growth)

R= Resistant (mild growth)

Table 5: Sensitivity of Gram positive and Gram negative isolated bacteria to water extract of *Eugenia caryophllata* determined by disc method

Bacteria species	Sensitivity to water extract		
	At concentration 10%	At concentration 20%	At concentration 30%
Staphylococcus aureus	+	++	++
Streptococcus pyogense	-	-	-
Streptococcus pneumoniae	-	-	-
Bacillus cereus	++	+++	+++
Corynebacterium renale	-	++	++
Pseudomonas spp	++	+++	+++
Klebsiella spp	-	+	++
E.coli	-	-	-
Salmonella spp	-	+	++

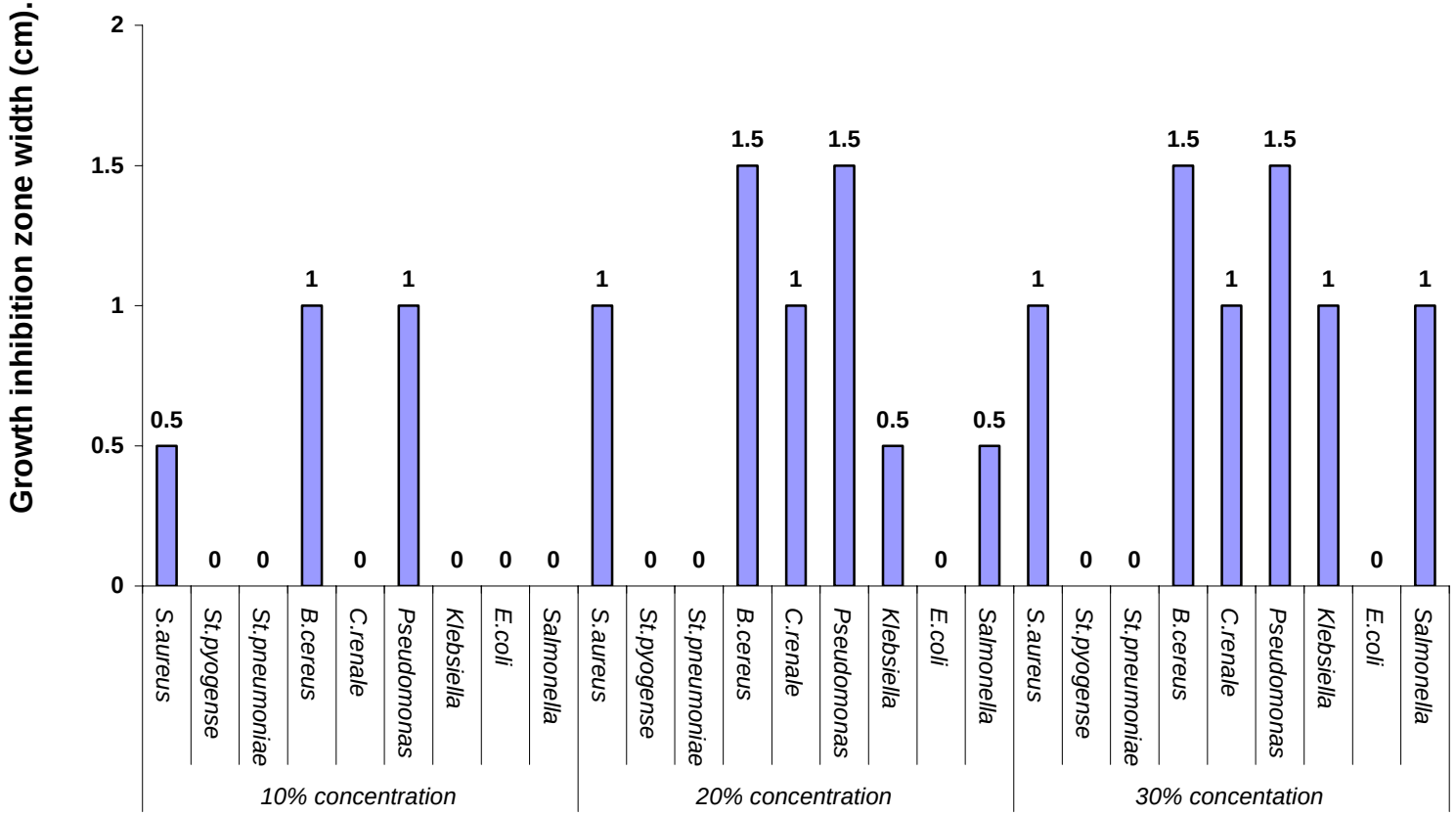
+++ Growth inhibition width= 1.5cm

++Growth inhibition width= 1cm

+ Growth inhibition width= 0.5 cm

- No growth inhibition zone

Fig. 2. Sensitivity of G +ve and G -ve isolated bacteria to water extract of *Eugenia caryophllata* determined by disc method.



3.3 Methanolic extraction :

3.3.1 Agar dilution method :

Methanolic extract of *Eugenia Caryophllata* was added to the medium to make three different concentrations of 10%, 20% and 30%. At all concentrations, all the tested organisms were inhibited except *Klebsiella spp* which showed a weak growth (Table 7).

3.3.2 Disc method :

The effect of methanolic extract of *Eugenia caryophllata* on tested organisms using disc method are shown in Table 8, Figure 5.

The 10% concentration of methanolic extract inhibited the growth of some bacterial species when examined by disc method and zone of inhibition measured 0.5cm for *Staphylococcus aureus*, *Corynebacterium renale*. *Bacillus cereus* and *Salmonella* while *Pseudomonas spp*, *Klebsiella spp*, *E.coli*, *Streptococcus pyogense* and *Streptococcus pneumoniae* were resistant and showed no zone of growth inhibition.

At 20% concentration the methanolic extract inhibited the growth of some bacteria examined. Inhibition zone width was 1cm for *Staphylococcus aureus*, *Corynebacterium renale*, *Bacillus cereus* and *Salmonella spp* while the growth of *Pseudomonas spp, Klebsiella spp*, *E.coli*, *Streptococcus pyogense* and *Streptococcus pneumoniae* and showed no zone of inhibition.

At 30% concentration methanolic extract inhibited the growth of some bacterial species examined and the zone width was 1cm for *Staphylococcus aureus*, *Corynebacterium renale*, *Bacillus cereus*, *Klebsiella spp, E.coli* and *Salmonella spp* while *Pseudomonas spp*,

Streptococcus pyogense and *Streptococcus pneumoniae* were resistant and showed no zone of inhibition.

3.3.3 Cup plate method :

The effect of methanolic extract of *Eugenia caryophllata* on tested organisms using cup plate method are shown in Table 9, Figure 6,7.

At concentration 10% of methanolic extract the zone of growth inhibition measured 0.5cm for *Pseudomonas spp*, *Staphylococcus aureus*, *Corynebacterium renale*, *Bacillus cereus*, *Klebsiella spp* and *Salmonella spp* while *E.coli*, *Streptococcus pyogense* and *Streptococcus pneumoniae* were resistant and showed no zone of growth inhibition.

At concentration 20% of methanolic extract the width of growth inhibition zone was 0.5cm for *Pseudomonas spp*, *Staphylococcus aureus*, *Corynebacterium renale*, *Bacillus cereus, Klebsiella spp* and *Salmonella spp* while the growth of *E.coli*, *Streptococcus pyogense* and *Streptococcus pneumoniae* were not affected and showed no zone of growth inhibition.

At concentration 30% of methanolic extract the zone of inhibition width was 1.5cm for *Pseudomonas spp* and *Bacillus cereus*, 0.5cm for *Staphylococcus aureus*, *Corynebacterium renale*, *Klebsiella spp, E.coli* and *Salmonella spp* while *Streptococcus pyogense* and *Streptococcus pneumoniae* were resistant and showed no zone of growth inhibition.

Table 6: Sensitivity of Gram positive and Gram negative isolated bacteria to water extract of *Eugenia caryophllata* determined by cup plate method

Bacteria species	Sensitivity to water extract		
	At concentration 10%	At concentration 20%	At concentration 30%
Staphylococcus aureus	+++	++++	++++
Streptococcus pyogense	-	-	-
Streptococcus pneumoniae	-	-	-
Bacillus cereus	+++	++++	++++
Corynebacterium renale	++	++++	++++
Pseudomonas spp	+++	++++	++++
Klebsiella spp	-	++	+++
E.coli	-	++	+++
Salmonella spp	-	+	+++

++++ Growth inhibition width= 2cm

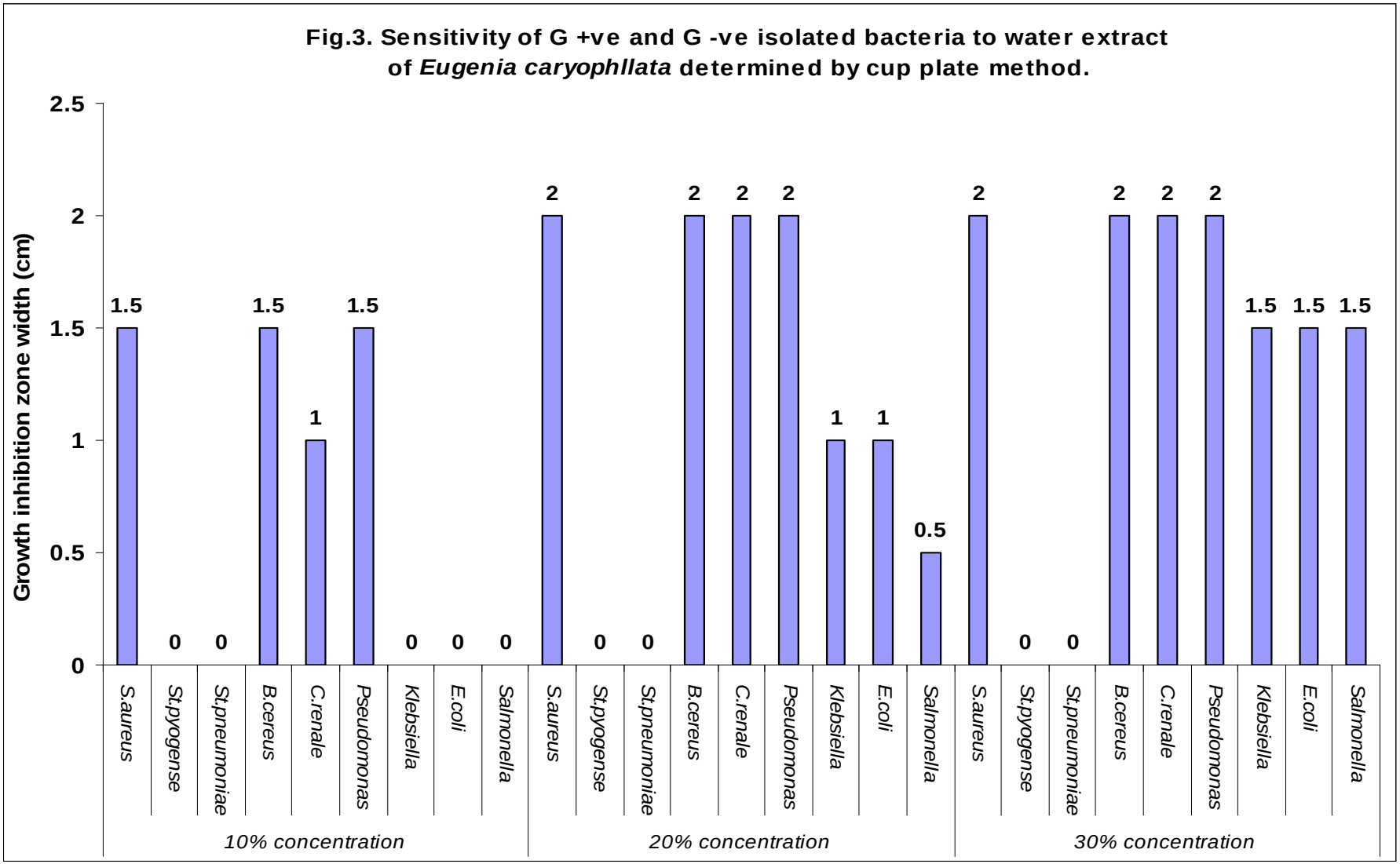
+++ Growth inhibition width= 1.5cm

++Growth inhibition width= 1cm

+ Growth inhibition width= 0.5 cm

- No growth inhibition zone

Fig.3. Sensitivity of G +ve and G -ve isolated bacteria to water extract of *Eugenia caryophllata* determined by cup plate method.



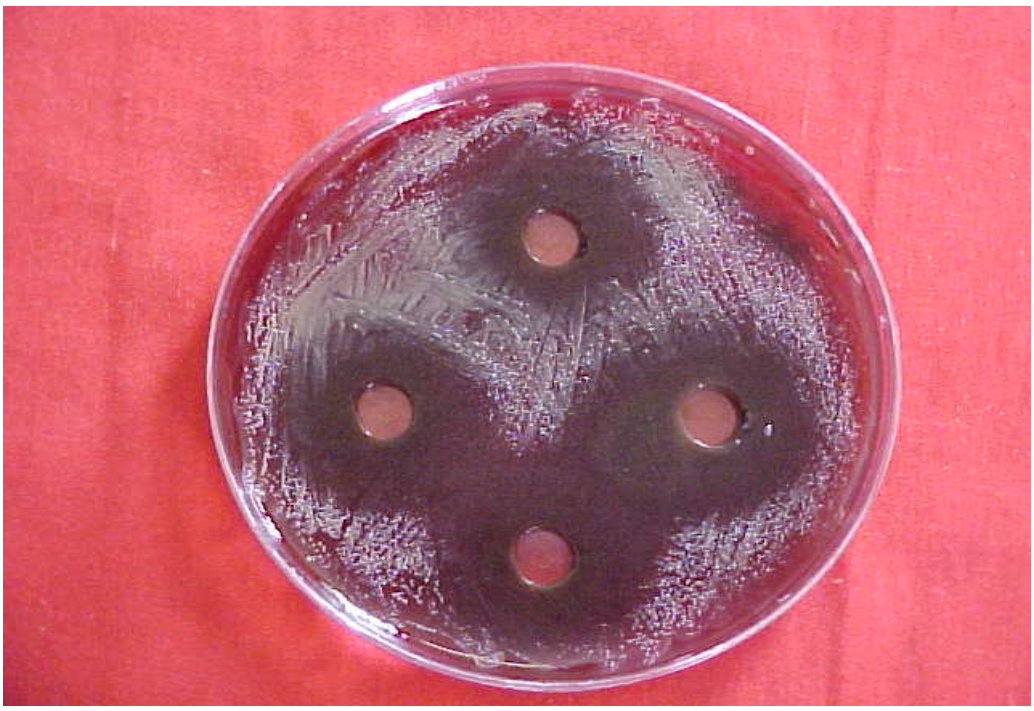


Figure.4. Sensitivity of *Staphylococcus aureus* to water extract of *Eugenia caryophllata* (20% concentration) determined by cup plate method.

Table 7: Sensitivity of Gram positive and Gram negative isolated bacteria to methanolic extract of *Eugenia caryophllata* determined by agar dilution method

Bacteria species	Sensitivity to methanolic extract		
	At concentration 10%	At concentration 20%	At concentration 30%
Staphylococcus aureus	S	S	S
Streptococcus pyogense	S	S	S
Streptococcus pneumoniae	S	S	S
Bacillus cereus	S	S	S
Corynebacterium renale	S	S	S
Pseudomonas spp	S	S	S
Klebsiella spp	R	R	R
E.coli	S	S	S
Salmonella spp	S	S	S

S= Sensitive (no growth)

R= Resistant (mild growth)

Table 8: Sensitivity of Gram positive and Gram negative isolated bacteria to methanolic extract of *Eugenia caryophllata* determined by Disc method

Bacteria species	Sensitivity to methanolic extract		
	At concentration 10%	At concentration 20%	At concentration 30%
Staphylococcus aureus	+	++	++
Streptococcus pyogense	-	-	-
Streptococcus pneumoniae	-	-	-
Bacillus cereus	+	++	++
Corynebacterium renale	+	++	++
Pseudomonas spp	-	-	-
Klebsiella spp	-	-	++
E.coli	-	-	++
Salmonella spp	+	++	++

++Growth inhibition width= 1cm

+ Growth inhibition width= 0.5 cm

- No growth inhibition zone

Fig. 5. Sensitivity of G +ve and G -ve isolated bacteria to methanolic extract of *Eugenia caryophllata* determined by disc method.

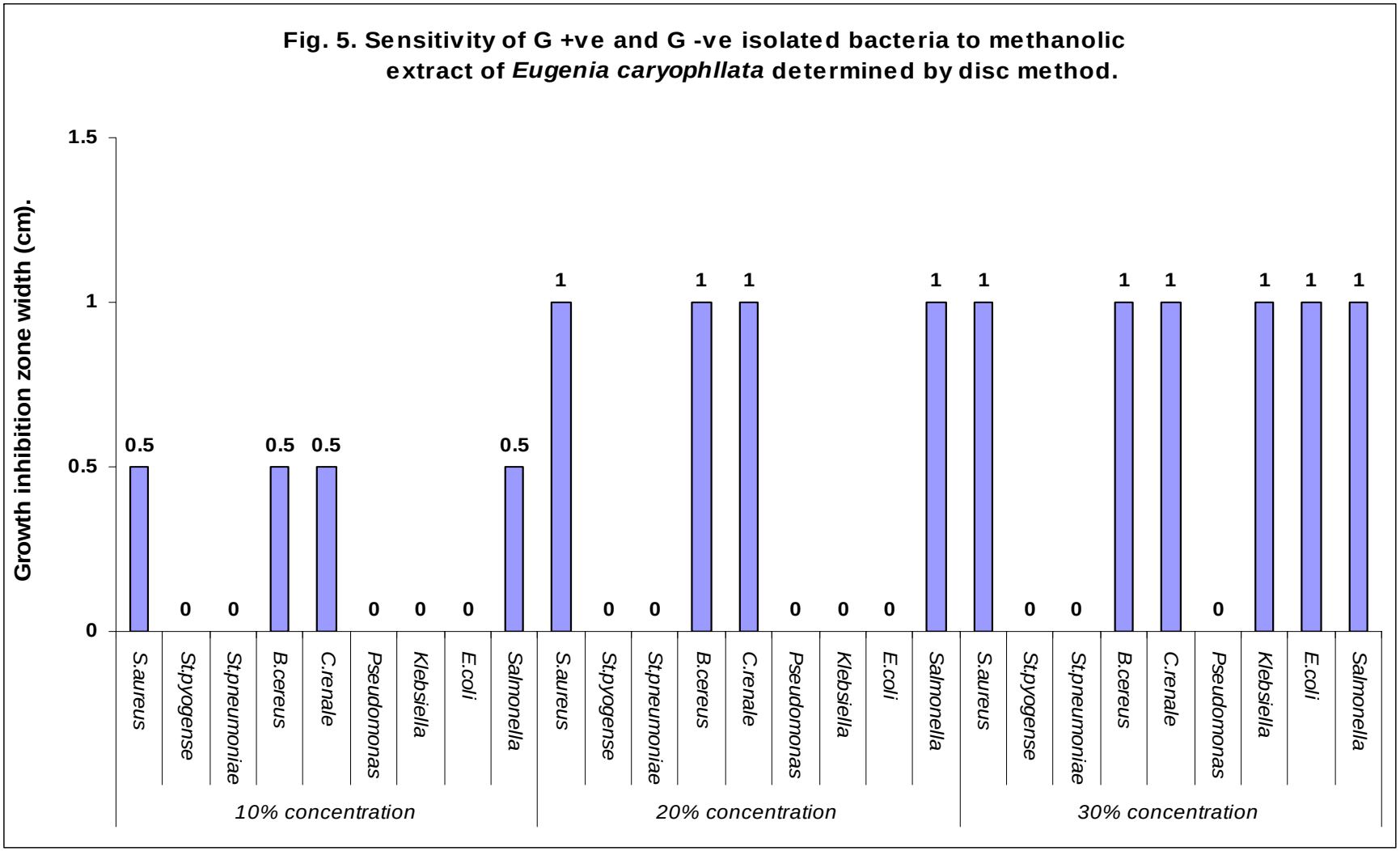


Table 9: Sensitivity of Gram positive and Gram negative isolated bacteria to methanolic extract of *Eugenia caryophllata* determined by Cup plate method

Bacteria species	Sensitivity to methanolic extract		
	At concentration 10%	At concentration 20%	At concentration 30%
Staphylococcus aureus	+	+	+
Streptococcus pyogense	-	-	-
Streptococcus pneumoniae	-	-	-
Bacillus cereus	+	+	+++
Corynebacterium renale	+	+	+
Pseudomonas spp	+	+	+++
Klebsiella spp	+	+	+
E.coli	-	-	+
Salmonella spp	+	+	+

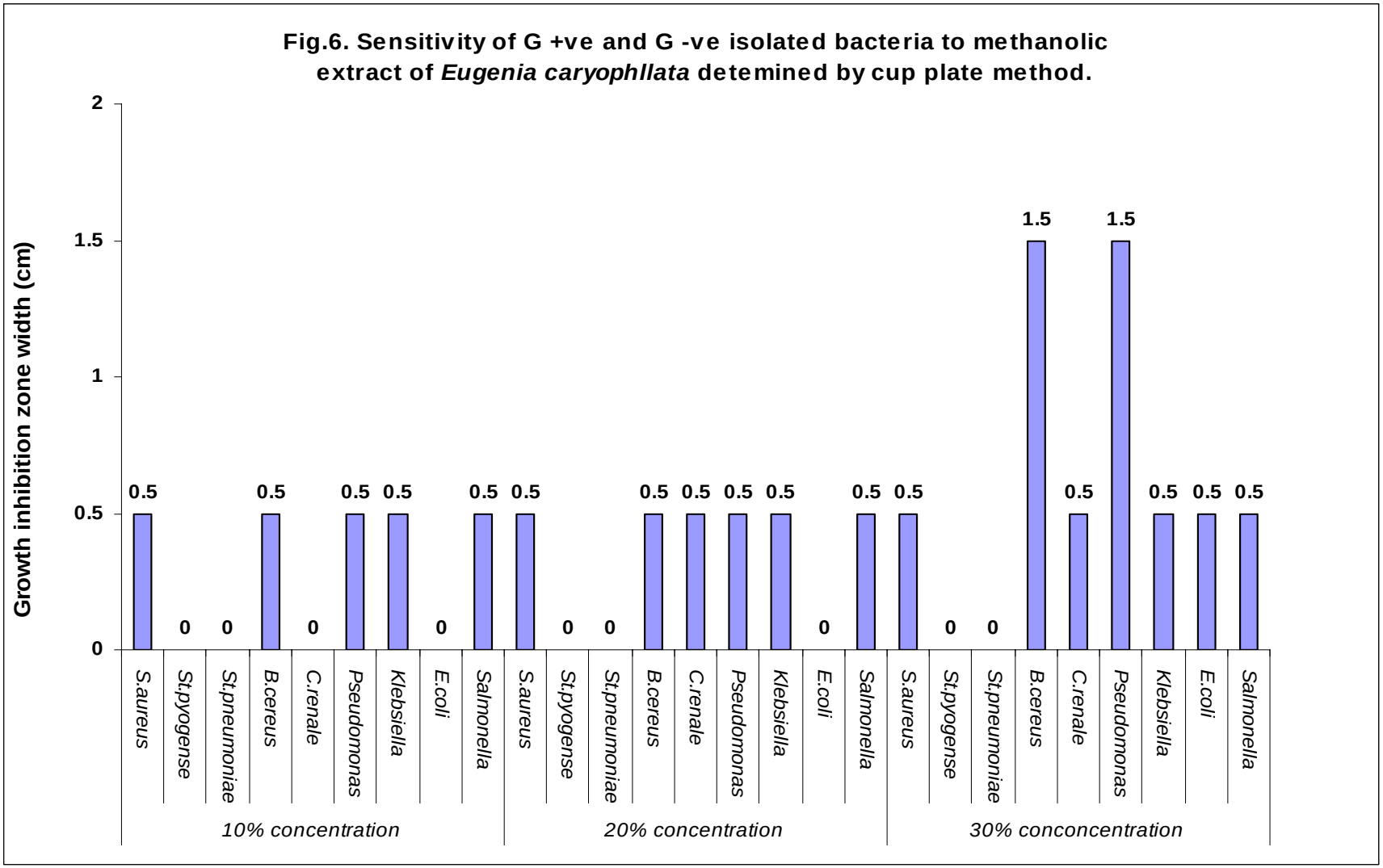
+++ Growth inhibition width= 1.5cm

++Growth inhibition width= 1cm

+ Growth inhibition width= 0.5 cm

- No growth inhibition zone

Fig.6. Sensitivity of G +ve and G -ve isolated bacteria to methanolic extract of *Eugenia caryophllata* detemined by cup plate method.



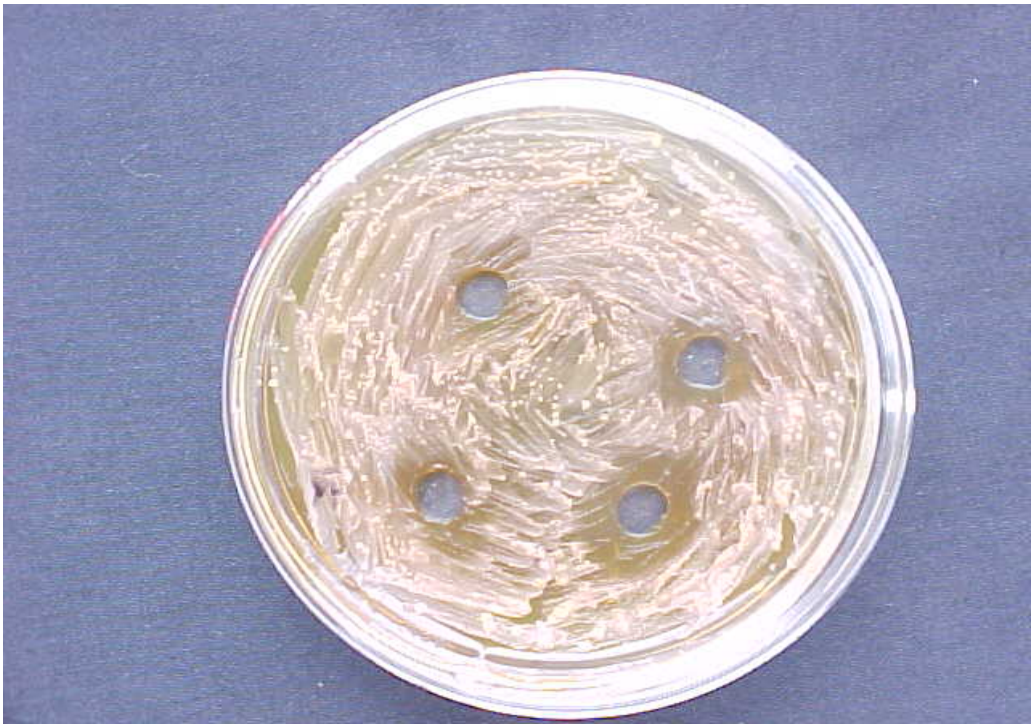


Figure 7. Sensitivity of *Bacillus cereus* to methanolic extract of *Eugenia caryophllata* (20% concentration) determined by cup plate method

3.4 Petroleum ether extraction :

3.4.1 Agar dilution method :

Agar plates containing the three different concentrations of petroleum ether extract of *Eugenia caryophllata* were prepared as described before.

The effect of petroleum ether extract of *Eugenia caryophllata* on growth of tested organisms using agar dilution method is shown in Table 10.

At all concentrations, the growth of the tested organisms were inhibited, except *Pseudomonas spp*, which showed good growth.

3.4.2 Disc method :

The effect of petroleum ether extract of *Eugenia caryophllata* on growth tested organisms using disc method is shown in Table 11, Figure 8.

At concentration 10%, the width of growth inhibition zone was 0.5cm for *E.coli*, *Klebsiella spp* and *Bacillus cereus* while *Pseudomonas spp, Salmonella spp*, *Corynebacterium renale*, *Staphylococcus aureus, Streptococcus pyogense* and *Streptococcus pneumoniae* were resistant and showed no zone of growth inhibition.

At concentration 20%, the width of growth inhibition zone was 0.5cm for *E.coli, Klebsiella spp* and *Bacillus cereus* while the growth of *Pseudomonas spp, Salmonella spp*, *Corynebacterium renale*, *Staphylococcus aureus*, *Streptococcus pyogense* and *Streptococcus pneumoniae* was not affected and showed no zone of growth inhibition.

At concentration 30%, the growth inhibition zone width was 1cm for *Bacillus cereus*, 0.5cm for *Klebsiella spp* and *E.coli* while the growth of *Pseudomonas spp*, *Salmonella spp*, *Corynebacterium renale*,

Staphylococcus aureus, *Streptococcus pyogense* and *Streptococcus pneumoniae* was not affected and showed no zone of growth inhibition.

3.4.3 Cup plate method :

The effect of petroleum ether extract of *Eugenia caryophllata* on growth of tested organisms using cup plate method is shown in Table 12, Figure 9 and 10.

At concentration 10 % the zone of growth inhibition width was 1cm for *E. coli*, *Klebsiella spp* and *Bacillus cereus* while the growth of *Pseudomonas spp, Salmonella spp, Corynebacterium renale, Staphylococcus aureus, Streptococcus pyogense* and *Streptococcus pneumoniae* was not affected and showed no zone of growth inhibition.

At concentration 20 %, the zone of growth inhibition width was 1cm for *E. coli*, *Klebsiella spp* and *Bacillus cereus* while the growth of *Pseudomonas spp, Salmonella spp, Corynebacterium renale, Staphylococcus aureus, Streptococcus pyogense* and *Streptococcus pneumoniae* was not affected and showed no zone of growth inhibition.

At concentration 30 %, the growth inhibition zone width was 2cm for *Bacillus cereus,* 1cm for *E.coli* and *Klebsiella spp* while *Pseudomonas spp*, *Salmonella spp*, *Corynebacterium renale*, *Staphylococcus aureus*, *Streptococcus pyogense* and *Streptococcus pneumoniae* were resistant and showed no zone of growth inhibition.

Table 10: Sensitivity of Gram positive and Gram negative isolated bacteria to petroleum ether extract of *Eugenia caryophllata* determined by Agar dilution method

Bacteria species	Sensitivity to petroleum ether extract		
	At concentration 10%	At concentration 20%	At concentration 30%
Staphylococcus aureus	S	S	S
Streptococcus pyogense	S	S	S
Streptococcus pneumoniae	S	S	S
Bacillus cereus	S	S	S
Corynebacterium renale	S	S	S
Pseudomonas spp	R	R	R
Klebsiella spp	S	S	S
E.coli	S	S	S
Salmonella spp	S	S	S

S= Sensitive (no growth)

R= Resistant (mild growth)

Table 11: Sensitivity of Gram positive and Gram negative isolated bacteria to petroleum ether extract of *Eugenia caryophllata* determined by disc method

Bacteria species	Sensitivity to petroleum ether extract		
	At concentration 10%	At concentration 20%	At concentration 30%
Staphylococcus aureus	-	-	-
Streptococcus pyogense	-	-	-
Streptococcus pneumoniae	-	-	-
Bacillus cereus	+	+	++
Corynebacterium renale	-	-	-
Pseudomonas spp	-	-	-
Klebsiella spp	+	+	+
E.coli	+	+	+
Salmonella spp	-	-	-

++Growth inhibition width= 1cm

+ Growth inhibition width= 0.5 cm

- No growth inhibition zone

Fig. 8. Sensitivity of G +ve and G -ve isolated bacteria to petroleum ether extract of *Eugenia caryophllata* determined by disc method.

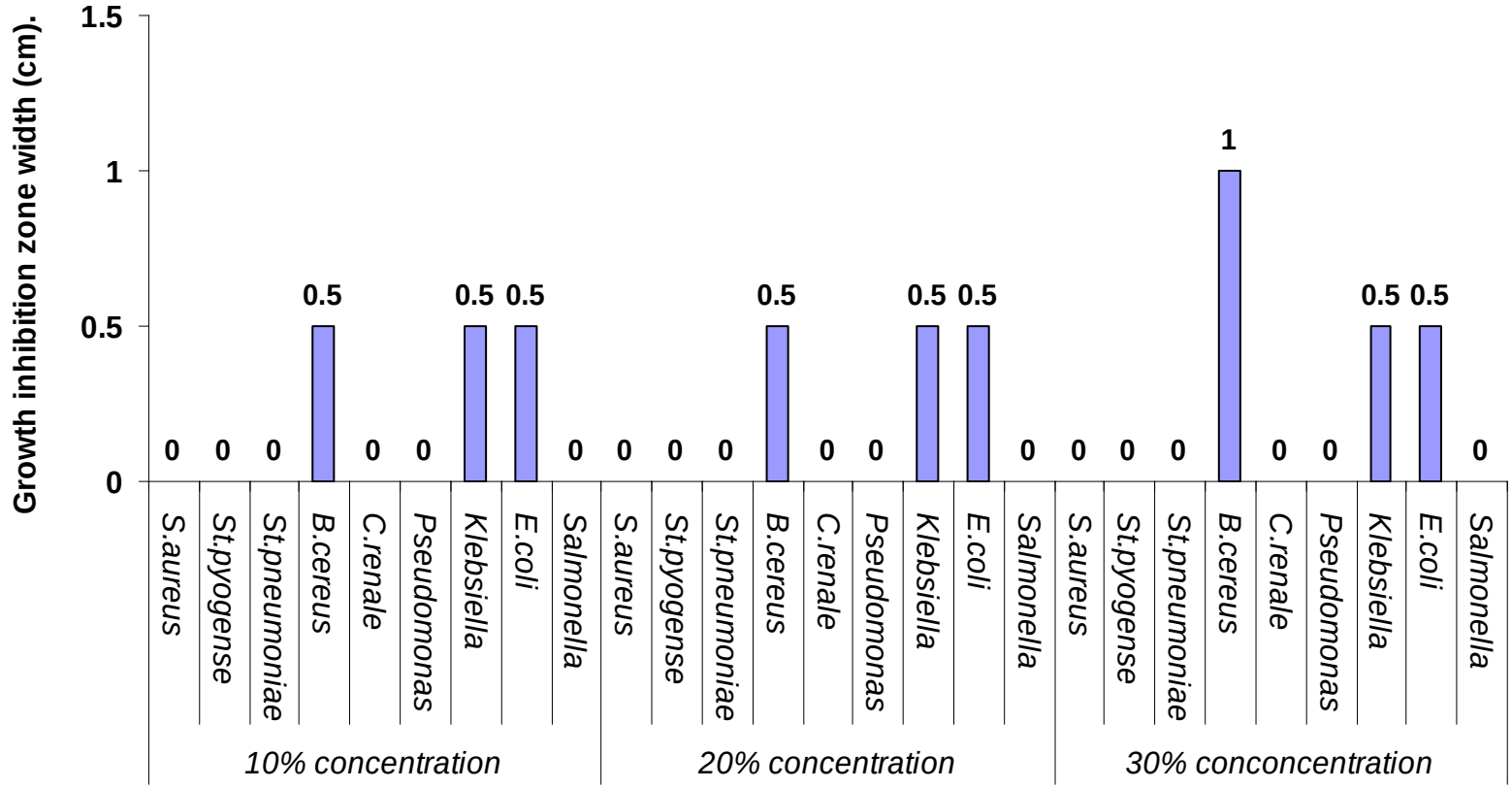


Table 12: Sensitivity of Gram positive and Gram negative isolated bacteria to petroleum ether extract of *Eugenia caryophllata* determined by cup plate method

Bacteria species	Sensitivity to petroleum ether extract		
	At concentration 10%	At concentration 20%	At concentration 30%
Staphylococcus aureus	-	-	-
Streptococcus pyogense	-	-	-
Streptococcus pneumoniae	-	-	-
Bacillus cereus	++	++	++++
Corynebacterium renale	-	-	-
Pseudomonas spp	-	-	-
Klebsiella spp	++	++	++
E.coli	++	++	++
Salmonella spp	-	-	-

++++ Growth inhibition width= 2cm

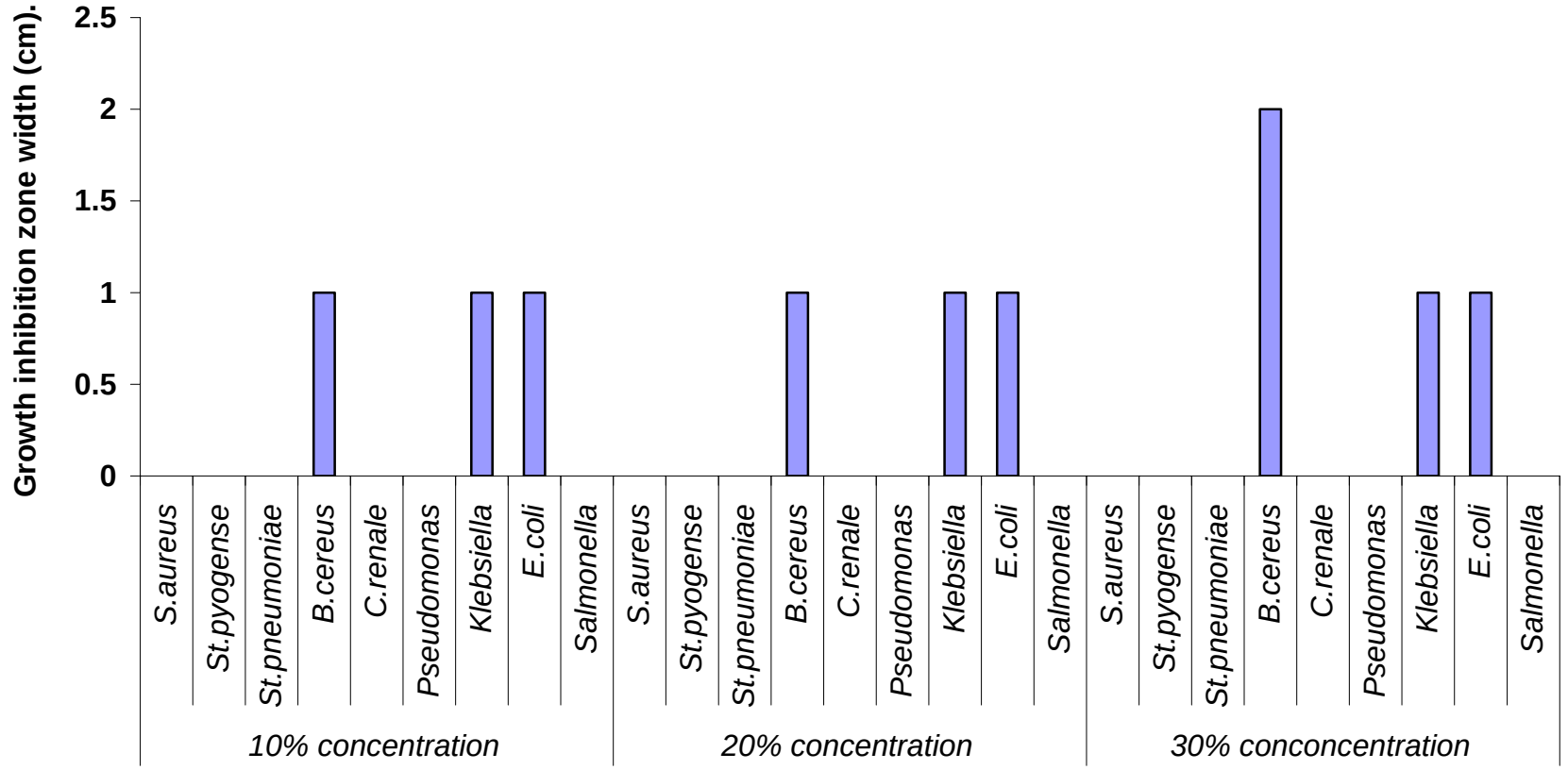
+++ Growth inhibition width= 1.5cm

++Growth inhibition width= 1cm

+ Growth inhibition width= 0.5 cm

- No growth inhibition zone

Fig.9. Sensitivity of G +ve and G -ve isolated bacteria to petroleum ether extract of *Eugenia caryophllata* determined by cup plate method.



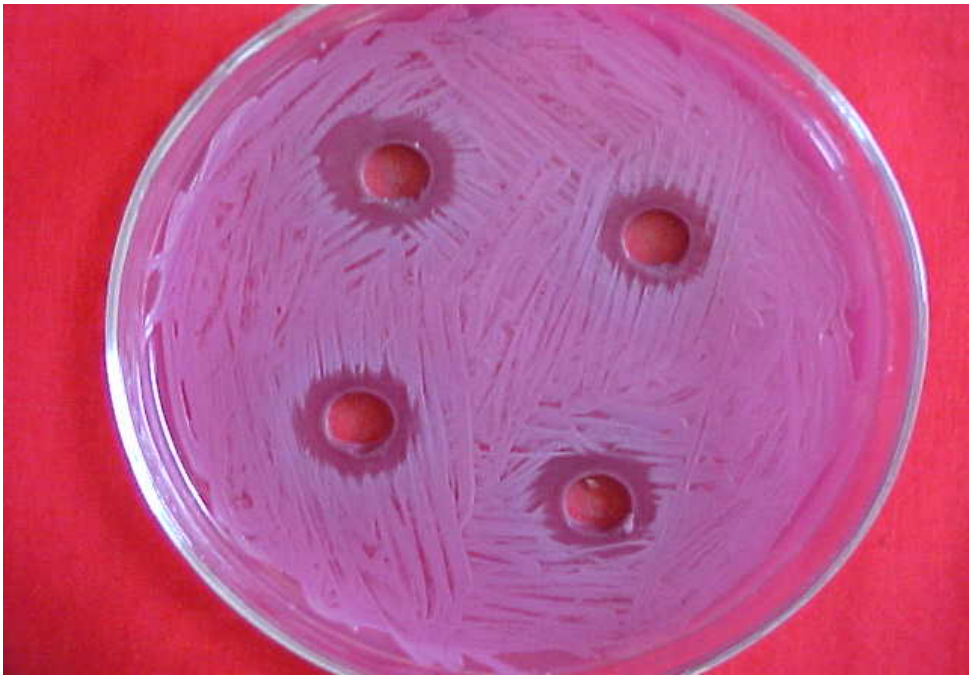


Figure 10. Sensitivity of *E.coli* to petroleum ether extract of *Eugenia caryophllata* (30% concentration) determined by cup plate method.

DISCUSSION

Some plants have been used for centuries as a treatment of infections and other illness in humans and animals. Some of them are believed having antimicrobial activities (Stockwell, 1988).

In the present study, the plant *Eugenia caryophllata*, which is believed amongst herbal therapists as antimicrobial agents, was examined. The possible *in vitro* effects of *Eugenia caryophllata* on the growth of isolated Gram positive and Gram negative bacteria were examined. Three different solvents, water, methanol and petroleum ether, were used for the extraction of soluble compounds, polar compounds and fatty compounds of the plant, respectively. The extracts were tested by three different methods, the agar dilution method, the impregnated filter paper method and the cup plate method.

In this study, the water extract of *Eugenia caryophllata* inhibited the growth of the isolated Gram positive and Gram negative bacteria when the three techniques were used.

The water extract of *Eugenia caryophllata* inhibited the growth of *S.aureus*, *B.cereus*, *C.renale* and *Pseudomonas spp* at all concentrations while *Klebsiella spp*, *E.coli* and *Salmonella spp* were inhibited at two concentrations, 20% and 30%. In the agar dilution method, the *Klebsiella* showed only mild growth at all concentrations, this revealed that *Klebsiella* was least sensitive organism to the antimicrobial activity of *Eugenia caryophllata* water extract.

However, the water extract of *Eugenia caryophllata* did not inhibited the growth of *St.pyogense* and *St.pneumoniae* at any concentrations. This shows, that the water soluble compounds of the

clove have inhibitory effect on the growth of *S.aureus, B.cereus, C . renale, Pseudomonas spp, Klebsiella spp, Salmonella spp* and *E.coli.*

The methanolic extract of *Eugenia caryophllata* inhibited the growth of *S.aureus, B.cereus. C.renale, Klebsiella spp, Pseudomonas spp*, *E.coli* and *Salmonella spp* when examined by impregnated disc method. However the growth of *B.cereus* and *C.renale* was inhibited by 10% and the higher concentrations. The methanolic extract of *Eugenia caryophllata* did not affect the growth of *St.pyogense* and *St.pneumoniae* at any concentrations examined. This indicates that the polar compounds of this plant have an inhibitory effect on growth of *S.aureus, B.cereus. C.renale, Klebsiella spp, Pseudomonas spp*, *E.coli* and *Salmonella spp*.

The petroleum ether extract inhibited the growth of *B.cereus Klebsiella spp* and *E.coli* at high concentration (30%) and showed a mild effect on growth of *Klebsiella spp* and *E.coli* at all concentrations. The petroleum ether extract did not affect the growth of *S.aureus*, *St.pyogense, St.pneumoniae*, *C.renale*, *Pseudomonas spp* and *Salmonella spp* when examined by the disc method. This revealed the fatty compounds of the plant have an inhibitory effect on growth of *B.cereus Klebsiella spp* and *E.coli*.

In this study, the effects of the water extract of *Eugenia caryophllata* on the isolated Gram positive and Gram negative bacteria was more effective than methanolic and petroleum ether extract. This indicates the water soluble compounds of *Eugenia caryophllata* have powerful antibacterial activity than the polar compounds of *Eugenia caryophllata*.

Also the present study showed that the methanolic extract of *Eugenia caryophllata* was more effective as antimicrobial agents when compared with the petroleum ether extract. This indicates that polar

compounds of this plant have stronger antimicrobial effect than the fatty compounds.

The water extract and methanolic extract of *Eugenia caryophllata* did not inhibit the growth of *Kebsella Spp* at any concentration (10%, 20% or 30%) when examined by agar dilution method. However, the growth of *Kebsella Spp* was inhibited by 20% and 30% water extract when examined by disc method and cup plate method. Also the growth of *Kebsella Spp* was inhibited by 30% methanolic extract when examined by disc method and by three concenteration (10%, 20% and 3%) of methanolic extract when examined by cup plate method. Remmal *et al*. (1993) reported that minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of clove methanolic extract for several bacterial species was lowered in the presence of agar. This may explain the growth of *Klebsiella* when ware and methanolic extract were examined by agar dilution method. Also it is possible that the active ingredient extracted by water and methanol is heat. Sensitive and was affected by agar temperature (50 ^{o}C) at the time of its addition to the molten agar.

In this study, it was also observed, the petroleum ether extract of clove inhibited the growth of all bacteria tested including *Klebsiella Spp*, but not *Pseudomonas spp*.

This may be because the petroleum ether extracted compound effective against *Pseudomonas spp* is heat sensitive and was affected by temperature of molten agar (55-60 ^{o}C) or its inhibitory activity was diminished in this presence of agar. The inhibition of *Klebsiella Spp* by petroleum ether extract of clove when examined by agar dilution method may be because petroleum ether extracted growth inhibitory compounds different from that extracted by water or methanol.

It was noticed in the present investigation that, *Streptococcus pyogenes* and *Streptococcus pneumoniae* were consiesantaly resistant to all concentrations (10%, 20%, 30%) of water, methanolic and petroleum ether clove extracts when examined by disc or cup plate method. This may be due to the presence of inhibitory compound or compounds of these extracts in concentration lower than minimum inhibitory concentration of (MIC). This need further investigation other bacteria examined in this study should viable responses to inhibitory activity of clove water, methanolic and petroleum ether extracts when tested by disc and cup plate method and the growth of some bacterial species were inhibited only by higher concentration of these extracts. Chromatographic fractionation revealed eight active compounds were isolated and identified from methanol extract of clove (Gai and Wu, 1996).

Water and petroleum extracts may contain different compounds and the concentration of active inhibitory compounds extracted may be different. This probably explains the viable response to inhibitory activity of clove water, methanolic and petroleum ether extracts.

In this study the cup plate method produced wider zone of growth inhibition than impregnated disc method when water extract (Table 5 V Table 6) methanolic extract (Table 8 V Table 9) and petroleum ether extract (Table 11 V Table 12) were examined. This reveals that the cup plate method is more sensitive than impregnated disc method for screening antibacterial activity of the clove extract. The agar dilution method is also a sensitive method when compared with impregnated disc method but it needed a ready prepared especial medium in which the plant extract is incorporated.

Similar to the previous studies (Valero and Kunicka, 2003; Carson *et al*. 1995, Deans and Syoboda 1989, Juven *et al*. 1993; Valnet 1982), this study reported antibacterial activity of clove against *Bacillus cereus*,

Pseudomonas aeruginosa and *Staphylococcus albus.* In addition, this study reported antibacterial activity of clove against *S. aureus, C.renale, Klebsiella spp, E.coli* and *Salmonella spp.*

Although this investigation did not examined the antibacterial of clove extracts against anaerobic bacteria, but other studies reported growth inhibitory effect of clove against *Clostridium perfringens*. Also other studies reported antimicrobial activity against *Diplococcus pneumoniae* and *Enterococci spp* (Carson *et al*, 1995, Deans and Syoboda, 1989, Juven *et al*. 1993 and Valnet, 1982).

The previous studies together with this study revealed that clove exhibits antibacterial properties against various bacteria isolated for animal and human sources.

Clove is generally considered safe, although a relatively small number of people may be allergic to eugenol (Zheng and Kenny, 1992).

The antibacterial activity of clove reported by this investigation and previous studies mentioned above and its relative safety for man, support the use of clove essential oils for treatment of injection caused by microorganisms (Kalemba and Kunicka, 2003). Previous study together with this finding showed that, clove exhibits broad antibacterial activities against bacteria supporting its traditional use as a treatment for diarrhoea and other digestive disturbances (Fetrow and Avila, 1991).

This study and previous ones support the believe that clove has antiseptic and antiseptic properties which reported by some dentists and patients that clove oil may relieve gum and tooth pain and may be useful as a topical antiseptic in mouth wash (Fetrow and Avila, 1991).

The antibacterial properties of clove reported by this study and previous ones explained the use of clove seeds and fruits for treatment of drinking water against E.coli in many developing countries where the

microbial quality of drinking water is a major public health problem (Blech *et al*,1991).

This preliminary study revealed growth inhibitory activity of clove water, methanolic and petroleum ether against several bacteria isolated from man and animals. Further studies are needed to valid this property, to fractionate the active compound of these extracts and to examine the inhibitory activity of each compounds separately.

Further work is needed on antimicrobial activity of *Eugenia caryophllata* since antibiotic drug resistance is now emerging as serous problem in this country.

Also became, this *in vitro* study shows, clove hold promise as beneficial anti bacterial treatment, further studies are necessary to determine if clove is non toxic and could be used for human.

Conclusions and Recommendations

Conclusions:

From the present study it can be concluded that:

1-*Eugenia caryophllata* is plant possessing antimicrobial effects against Gram positive and Gram negative bacteria *in vitro*.

2-The water and methanolic extract of *Eugenia caryophllata* are more effective found antimicrobial agent than the petroleum ether extract.

3-The cup plate method is a simple method and it give clear results.

Recommendations:

1-Further testing of water and methanolic extracts of *Eugenia caryophllata in vivo* on Gram positive and Gram negative bacteria should be camed out.

2-Fractionalization of water, methanolic and petroleum ether extracts of *Eugenia caryophllata* to identify their active ingredients.

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